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GROWTH

A JOURNAL FOR STUDIES OF DEVELOPMENT AND INCREASE

VOLUME IV, NUMBER 2

PAPERS 126-134

AUGUST, 1940 $5.00 A YEAR

GROWTH

A journal for the coordination of studies of increase and development as general properties of nature. Published by contributors and subscribers on a cooperative, non-profit, non-salary basis. The field of investigation is primary and work will be published in which the approach is through mathematics, physics, chemistry, genetics, embryology, philosophy and anthropology, just as will studies of development in its several expressions, and of mass increase and the part played therein by nutrition, vitamines and hormones.

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All papers for publication are to be sent to the same address, *The Editors of Growth, Dairy Building, Cornell University, Ithaca, N. Y.*

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THE EFFECT OF CENTRIFUGATION ON HYDRANTH AND STOLON REGENERATION AND GROWTH IN OBELIA GENICULATA

By

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(*Received for publication February 20, 1940*)

The physical factors of growth are self-evident. What part they play in each separate aspect of developmental expression has yet to be evaluated. The pioneer work of Goldfarb (1906) and Torrey and Martin (1910) on the influence of light on regeneration in hydroids; of Loeb (1925) with gravity; and of Gurwitsch with mitogenetic rays (Rahn, 1936), are indicia of the possibilities in this direction. The situation with respect to regeneration is reviewed by Millot (1931).

Interest in the differential expression of the several basic activities of developmental growth, and a desire to extend observation from the role of 'substance' to the role of 'state' (Hammett, 1938) prompted the present inquiry into the effect of centrifugal force on these phenomena as exhibited in *Obelia geniculata*.

PROCEDURE

Freshly collected colonies were used.

For each experiment the colonies were collected into fresh sea-water. Changes into fresh sea-water were frequent during the necessary manipulations. Careful selection provided matched series of 10 pairs composed of colonies of like numbers of hydranth-bearing pedicels of from 8 to 13 members. In a goodly number of experiments there were 10 control colonies of like composition. This gave three groups of colonies of approximately equal growth potentiality. Each colony which was to be centrifuged was carefully floated in fresh sea-water into a 10 cm. piece of 3mm. glass tubing by suction through an attached and removable piece of clean pure gum rubber tubing. By pulling the colony into the glass tube proximal end up distortion and breakage were avoided. The colony was held in place by capillarity and gentle contact. This procedure kept the colony oriented in the lines of force and prevented slumping to the bottom of the centrifuge tube. The 20 specimens thus prepared were divided into 2 groups, one of each pair in each group, and put into 50 ml. pyrex centrifuge tubes with enough fresh sea-water to cover. One group contained colonies with the distal ends

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towards the center: the other with these towards the periphery. The set-up was then centrifuged for 90 minutes, unless otherwise stated, at either 790 or 1060 R.C.F. After this the colonies were transferred from the little glass tubes to finger-bowls containing fresh sea-water where they stayed for the rest of the 24-hour period of experiment. During this time three changes into fresh sea-water were made. The controls were kept in finger-bowls throughout and had the same number of changes into fresh sea-water as the centrifuged colonies. At the end of 24 hours record was made of regenerative and other growth changes in each colony.

Two sets of observations were made: one of stolon production and growth from the cut proximal end of the hydrocaulus; and one of hydranth regeneration and development after removal of the distal animal. Mindful of Davenport's (1896) early finding that "- - - definiteness of the result (regeneration) is in Obelia a function of the distance of the cut edge from the base of the stem" and "- - - that, in nature, regeneration takes place more frequently from the more distal than from the more proximal levels", care was taken to make equivalent cuts on each pair or triad of colonies.

The numbers of hydranths and stolons so produced are recorded in Tables I and II. The analysis is made from the percentage relations of centrifuged to uncentrifuged specimens; and the percentage relations of growths emanating from peripherally placed cut ends to those arising from the centrally placed. These are recorded at the bottom of the Tables. The first series gives the combined data and their derivatives: the second (A) those attained after high speed: and the third (B) those resulting from low speed. The percentages are quotients of averages.

All glassware was cleaned in bichromate-sulfuric acid mixture and washed 80 times in tap water and 10 in distilled. Just before use it was rinsed 5 times in fresh sea-water.

RESULTS

Stolons.

All colonies did not produce stolons. The stolon/colony ratios are given in the first 3 columns at the bottom of Table I. Moderate centrifugation had no great influence on stolon production as shown by the numbers and ratios of the B series. More rapid speed may have been slightly detrimental. The A values suggest this and indicate that stolon production from the peripherally placed cut ends was better than from the centrally placed.

Stolon growth in length, whether it be calculated on the basis of colony potential or actual growth was retarded. The quotient of centrifuged to control length is less than 100 in all summations. More rapid

TABLE I

EFFECT OF CENTRIFUGATION ON STOLON PRODUCTION AND GROWTH IN OBELIA GENICULATA

	Number of Colonies			Number of Stolons			STOLONS TOT. Av. L. — Colony No.			TOT. Av. L. — Stolon No.			METAPLASIA Bud			1/2			3/4			
Expt.	D	C	U	D	C	U	D	C	U	D	C	U	D	C	U	D	C	U	D	C	U	D.S.
							A		1060	R. C. F.												
1	10	—	10	10	—	10b	25.8	——	29.1	25.8	——	29.1	0	—	0	0	—	0	0	—	0	0
2	10	—	10	10	—	10	28.3	——	35.1	28.3	——	35.1	0	—	0	0	—	0	0	—	0	0
3	10	—	9	10	—	9	32.3	——	32.4	32.3	——	32.4	0	—	0	0	—	0	0	—	0	0
4	10	—	10	10	—	9	34.2	——	31.7	34.2	——	35.2	0	—	1	0	—	0	0	—	0	0
5	10	—	10	10	—	10	28.2	——	19.6	28.2	——	19.6	0	—	0	0	—	0	0	—	0	0
6	10	10	9	9	8	8	19.9	25.1	18.3	22.2	31.4	22.9	1	1	1	0	0	0	0	0	0	0
7	10	10	10	7	9b	8	20.0	28.6	23.0	28.6	35.6	28.7	0	0	0	0	0	1	0	0	0	0
8	10	10	10	10b	10	9	28.4	37 2	26.8	28.4	37.2	29.8	2	1	0	2	2	3	0	1	0	C
9	10	10	8	8	9	8	19.0	31.3	31.8	23.8	34.8	31.8	2	0	0	1	2	3	2	1	0	C
10	10	10	10	9	10	6	26.0	28.9	17.6	28.9	28.9	29.3	0	1	0	2	0	0	0	0	0	0
11	10	10	10	9	10	10	28.9	28.9	30.6	32.1	28.9	30.6	1	1	1	0	0	0	0	0	0	0
12	9	10	10	9	10	9	36.2	37.2	34.2	36.2	37.2	38.0	0	0	0	1	0	0	0	0	0	D
							B		790	R. C. F.												
13	10	10	10	7	10	10b	21.4	31.3	30.9	30.6	31.3	30.9	0	0	0	1	0	0	0	0	0	0
14	10	10	10	9	9	10	23.6	25.2	24.1	26.2	28.0	24.1	0	0	0	0	0	0	0	0	0	0
15	10	10	10	10	10	9	33.2	34.9	29.1	33.2	34.9	32.3	0	0	0	0	0	0	0	0	0	0
16	10	10	10	10b	10	10	35.6	39.3	33.7	35.6	39.3	33.7	0	0	1	0	0	0	0	0	0	0
17	10	9	10	10	9	10	33.5	37.7	34.3	33.5	37.7	34.3	1	0	0	0	0	0	0	0	0	C
18	10	10	10	10	10	10b	15.8	15.9	16.4	15.8	15.9	16.4	0	0	0	0	0	0	0	0	0	U
19	10	10	10	10	10	10	17.5	17.0	18.3	17.5	17.0	18.3	0	0	0	0	0	0	0	0	0	U
20	10	10	10	10	10b	10	30.7	32.7	30.6	30.7	32.7	30.6	0	0	0	0	0	0	0	0	0	0
21	10	10	10	10	10	9	31.4	30.1	27.2	31.4	30.1	30.2	0	0	0	0	0	0	0	0	0	0
22	10	10	10	10	10	10	27.0	24.4	31.1	27.0	24.4	31.1	0	0	0	0	0	0	0	0	0	U
23	10	10	9	10	10	9	30.1	34.1	26.3	30.4	34.1	26.3	0	0	0	0	0	0	0	0	0	0
T	229	179	225	217	174	213	27.3	30.0	27.5	28.7	31.1	29.2	7	4	4	7	4	7	2	2	0	7
%	95	97	95	98		96	91		92	92		94	137		78	137		137	78		—	
Gr					102			99			98			175			100			—		
A	119	70	116	111	66	106	27.3	31.0	27.5	29.1	33.4	30.2	6	4	3	6	4	7	2	2	0	4
%	93	93	91	98		94	88		89	87		90	88		44	88		102	58		—	
Gr					105			99			96			200			86			—		
B	110	109	109	106	108	107	27.3	29.3	27.5	28.4	29.6	28.0	1	0	1	1	0	0	0	0	0	3
%	96	99	98	98		99	93		94	96		95	—		—	—		—	—		—	
Gr					99			99			101			100			—			—		

D—Stolons peripheral: C—Control: U—Stolons central.
Expt. 1—75 minutes: Expts. 4 & 5 180 minutes.
b—One broken and not included D. S.—Double stolon.
Gr — Growth

speed determined a greater inhibition. The A quotients were less than the B. There is no evidence of differential reaction to directional difference in centrifugal force. Stolon growth from peripherally placed cut ends was no more and no less than that from the centrally placed,

Centrifugation did, however, act to forward metaplasic development of new hydranths on the peripherally directed stolons. It did not affect expression of this activity on the centrally directed unless to retard. The break-down data given in the last columns at the bottom of Table I can be combined into one figure for each set which gives a value expressive of relative developmental accomplishment in each. By giving a credit of 1 to bud formation, 2 to half-stage growth, and 3 to attainment of the three-quarter stage, it is found that metaplasic growth in peripherally directed stolons was to that of the centrally directed as 27 is to 18; while metaplasia in the centrally directed was to that of the adjusted control as 18 is to 20.

There were three double stolons from the centrally placed cut ends, one from the peripheral, and three from the controls.

Hydranths.

In a few experiments record was made of new hydranth production and growth from uncut distal ends. This was not regenerative but natural colonial growth. More new hydranths were produced in the peripherally directed tips. Thus—potentiality was expressed to 33 per cent in these and to but 24 per cent in the centrally directed. Potentiality expression is the quotient of colony number into new hydranth number. The developmental accomplishment of these new growths was also greater in the peripherally than in the centrally directed. As seen from Table II, and using the same index as was used in evaluating stolon metaplasia, the relation of the former to the latter was as 29 is to 18.

Turning now to hydranth regeneration and growth after removal of the distal member of the colony by cutting, similar reactions are found. From the data of Tables I and II it is found that at 790 R.C.F. potentiality for hydranth production by regeneration was expressed to 58 per cent in peripherally directed tips, and to 47 per cent in centrally: that at 1060 R.C.F. it was 74 per cent and 71 per cent respectively: and that when the experiments of both series are combined it was 62 and 53 per cent. An inhibition of regeneration expression in the centrally directed tips is suggested by the fact that potentiality expression of centrifuged specimens tends to be less than that of uncentrifuged controls in both the B series and the totals, while potentiality expression of the peripherally directed tends to be greater.

The higher value at higher speed is not to be taken as due to 1060

TABLE II

EFFECT OF CENTRIFUGATION ON HYDRANTH REGENERATION AND GROWTH IN OBELIA GENICULATA

Expt.	Top Growth Bud D	C	U	Half D	C	U	HYDRANTH Regeneration Bud D	C	U	Half D	C	U	3/4 D	C	U	Complete D	C	U	TOTAL Progression D	C	U
									A 1060 R. C. F.												
2	2	—	3	3	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	1	—	1	1	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	4	—	2	2	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5	3	—	3	2	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6	1	3	0	1	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	0	0	1	0	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	2	1	2	6	4	3	0	2	2	1	2	0	18	21	14
9	—	—	—	—	—	—	1	2	3	4	4	3	1	1	0	1	1	0	16	17	9
10	—	—	—	—	—	—	1	3	2	4	2	5	0	3	0	1	0	1	13	16	16
11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	1	0	1	3	2	2	3	4	3	0	2	0	16	24	14
									B 790 R. C. F.												
13	—	—	—	—	—	—	2	0	2	2	5	7	3	4	0	0	0	0	15	22	16
14	—	—	—	—	—	—	1	1	1	1	4	3	3	1	3	0	0	0	12	12	16
15	—	—	—	—	—	—	2	2	3	3	3	1	0	1	1	0	0	0	8	11	8
16	—	—	—	—	—	—	0	2	2	3	2	1	1	1	1	0	1	1	9	13	11
17	—	—	—	—	—	—	2	2	2	5	1	3	0	2	0	0	0	0	12	10	8
18	—	—	—	—	—	—	7	6	4	0	0	0	0	0	0	0	0	0	7	6	4
19	—	—	—	—	—	—	6	2	3	3	2	0	0	0	0	0	0	0	12	6	3
20	—	—	—	—	—	—	2	2	1	2	1	1	1	0	1	0	0	0	9	4	6
21	—	—	—	—	—	—	3	2	4	2	0	2	0	0	0	0	0	0	7	2	8
22	—	—	—	—	—	—	2	1	2	2	2	0	1	0	0	0	0	0	9	5	2
23	—	—	—	—	—	—	2	3	1	1	1	1	2	0	0	0	0	0	10	5	3
T	—	—	—	—	—	—	34	29	33	41	33	32	15	19	11	3	6	2	173	176	138
%	—	—		—	—		117		114	124		97	79		58	50		33	98		78
Gr	—			—			103			128			136			150			125		
A	11	3	10	9	0	4	5	6	8	17	12	13	4	10	5	3	5	1	61	80	53
%	122		111	—		—	83		133	142		108	40		50	60		20	79		66
Gr	110			225			63			131			80			300			119		
B	—	—	—	—	—	—	29	23	25	24	21	19	11	9	6	0	1	1	110	96	85
%	—	—		—	—		126		109	114		90	122		67	—		100	115		89
Gr	—			—			116			126			183			—			129		

D — Distal end peripheral; C — Control; U — Distal end central.
Gr — Growth.

R.C.F. Control colonies as well as test are more active in this respect as shown by the fact that hydranth regeneration in the former jumped from 50 per cent expression in the B series to 83 in the A. A moral might here be printed regarding the necessity for controls. If such had not here been run simultaneously the higher regeneration expression at higher speed might have contributed to an interpretative fallacy.

Moderate speed did not impair the regenerative faculty. High speed centrifugation was detrimental here as in stolon growth. This is shown by the fact that test/control quotients of hydranth production in the A series are well under 100. On the same basis it also tended to hold back developmental accomplishment as shown in the last brackets of Table II.

Development of regenerated hyranths was enhanced in peripherally directed colonies. Using the same system of computation as was employed for metaplasic growth and adding thereto a credit of 4 for attainment of a complete hydranth it is seen in the last brackets of Table II that development of hydranths from peripherally directed cut ends was to that from the centrally directed as 110 is to 85 in the B series; as 63 is to 53 in the A series; and as 173 to 138 when all experiments are combined.

Development of regenerated hydranths was held back in the centrally directed colonies. This is suggested in the A series by the fact that growth in this group was less than control to greater extent than in the peripherally directed group: and demonstrated in the B series by the fact that growth of the centrally directed group was but 89 per cent of the control while that of the peripherally directed was 115 per cent.

This is supported by the fact that the quantitative disparity between the two groups increases with increase in developmental accomplishment.

DISCUSSION

Two things are to be kept in mind. First that the data represent growth *subsequent* to centrifugation, not growth occurring during centrifugation. And second that two distinct aspects of growth are recorded, viz. new growth production: and growth progression of the new structures.

It was surprising to find the mechanisms responsible for stolon production and growth in length essentially indifferent to directional application of centrifugal force. Reflection shows what observation might have taught that this is not necessarily contrary to nature. For there is no evidence in nature that obelia stolon growth is geotropic. In fact stolons grow up or down or laterally with apparently equal facility. They do not have the geotropic characteristics of many plant roots. They spread over surfaces apparently without regard to the direction of gravitational force. This is not the curling or haptotropism of plants (Boysen Jensen, 1939) but something quite different. For lack of a better term it may perhaps be called planotropism, or surface tropism. Other marine organisms exhibit the property.

The result is somewhat different than that reported by Schechter (1934; 1935) who found centrifugation acted to induce shoots in the red alga *Griffithsia bornetiana*. Apparently the nature of the organism is primary. This is consistent with the postulate that physical forces and the reactions thereto are determined by the nature of substance (Hammett, 1936). "The Sun's Light when he unfolds it Depends on the Organ that beholds it." Wm. Blake (1793).

In contrast to the relative unresponsiveness of stolon length growth is the reaction of stolon metaplasia wherefrom a hydranth instead of more stolon is produced from the distal stolon cells. Here, as shown in Table I, centrifugation acts to stimulate metaplasic activity; more hydranths appear on the ends of the stolons subjected to the outward pull.

This difference shows that separate aspects of growth expression in one and the same organism are separately and differently reactive to one and the same physical stimulus even though each has common com-

ponents. Again it is clear that 'substance' is a primary determinant of reaction while 'state' is secondary.

There is a certain amount of data from experiments with *Antennularia antennina* and *Endendrium racemosum* as reported by Millot (1931) which suggest pressure as a deciding factor in this heteromorphosis.

If it be granted that chemical specialization of cellular substance is the basis of that differentiation which produces cells which deviate in composition, form, and function from parent cells (Hammett, 1936), then metaplasia and heteromorphosis are in large part expressions of such chemical specialization or differentiation. It is interesting to wonder in passing if the differentiation of callus reported by White (1939) when he pushed his tissue cultures down under his liquid nutrient medium instead of letting them grow on top of a semi-solid agar, might perhaps be contributed to by the increased pressure quite as much as by the change in available oxygen.

The suspicion of a forwarding influence of properly applied pressure on differentiation is not weakened by other results. Perhaps it had no effect on stolon length growth because once this is begun there is no differentiation if the growth remains that of stolon formation.

If we abide by our definition of differentiation as a cellular chemical specialization it follows that differentiation is a necessary fore-runner of anlagen formation for new hydranth production. For the cells of the pedicel from which the new animal is to come certainly become specialized for that function as the growing pedicel becomes ripe for its expression. And pressure—as applied through centrifugation—forwards the *subsequent* production of hydranths from these potential sites of anlagen formation.

Adherence to our definition yields the belief that differentiation is also a participant in the cellular changes incident to the reconstitution phase of regeneration. Certainly chemical specialization must occur before the cells capable of initiating the new hydranth become oriented for that expression. And pressure—as applied through centrifugation —immediately after the removal, forwards the *subsequent* production of a new hydranth by regeneration. Not only does this happen, but what is of added significance, fewer hydranths were produced by regeneration when not pressure, but release or a somewhat relaxation from pressure is afforded.

Turning from hydranth production to hydranth developmental growth it is found that although proliferation, differentiation, and organization expression were speeded up by the centrifugal push and somewhat retarded by the centripetal pull, no one of these develop-

mental activities was favored over the others. Nor should such have been expected since the centrifugal force was not applied specifically during the exercise of any single activity.

It is easy enough to say that the hydranths grew better because they got an earlier start but that does not explain how it happened.

TABLE III

PERCENTAGE GROWTH PROGRESSION FROM ONE DEVELOPMENTAL STAGE TO THE NEXT OF THE OBELIA HYDRANTHS OF TABLES I AND II

Series Set	TOTALS D	C	U	Series A D	C	U	Series B D	C	U
Expts. 1—23				METAPLASIA					
Bud to 1/2	56	60	63	57	60	70	50	0	0
1/2 to 3/4	22	50	0	33	50	0	0	0	0
Expts. 2—7				NATURAL					
Bud to 1/2	—	—	—	45	0	29	—	—	—
Expts. 8—23				REGENERATION					
Bud to 1/2	63	67	58	83	82	70	54	57	51
1/2 to 3/4	31	23	29	29	56	31	31	32	27
3/4 to Complete	17	24	15	43	53	17	0	10	14

The percentage increment from one stage to the next was pretty consistently greater in the set with distal ends peripheral. Particularly in the A series. This is shown in Table III. Other things being equal, approximately equal percentage progression should be exhibited regardless of any early or late start. That such did not obtain and that the growth reaction was in the same direction as the initiation indicates the change due to centrifugation persists into the later appearing developmental activities.

It is the obligation of every scientist to posit an interpretation of the data he reports. Through this he not only attempts to coordinate his results but also to indicate the lines of further inquiry and testing. We have here to tie together the stolon indifference to centrifugation; the possible particular influence of centrifugation on differentiation; and the persistence of the growth forwarding influence in hydranth development subsequent to its exhibition at the initiation stage. For clarity of exposition the last may be first.

Hammett and Padis (1935) observed that "when developmental growth induces anabolism (in Obelia hydranths) the current of hydroplasmic granules is out into the growing part". This has been made a matter of cinematic record. Chapman (1937) records the accumulation of SH where active hydranth proliferation is taking place. This is correlatable with Coldwater's (1933) association of SH with regenerative growth. Hammett and Chapman (1938) made chemical tests for free amino acids in general and for amino acids of particular growth function in particular on living colonies of *Obelia geniculata* as taken directly from their natural habitat and after longer or shorter periods

of feeding in amino acid containing culture solutions. The tests showed amino acid concentration both in solution and on granules in the distal hydroplasm immediately contiguous to the growing parts. These data yield, for this organism at least, indisputable evidence that the hydroplasm is a carrier of specific growth significant substances to the developing hydranths.

It is therefore logical to conclude that centrifugation induces a considerable piling up of these in the termini of stolons and pedicels wherefrom the hydranths are to spring. This accumulation could well contribute to increased hydranth and stolon* production just as SH increase contributes to increased cellular production, and auxin concentration to increased root production (Boysen Jensen, 1939). And the exercise of a pull in the opposite direction could have the opposite effect, viz. a lesser hydranth production.

Now the data of Hammett and Chapman (1938) show not only that the growth substances are concentrated in the distal hydroplasm, but that they also are passed on from this into the cells of the part which is growing and actually concentrated therein.

It is therefore logical to conclude that this process too is forwarded by centrifugation (Prát, 1934). And it is not beyond reason to assume that once the period of centrifugation is over and the colonies are allowed to pursue their growth undisturbed, this luxus supply of growth essentials and regulators (Hammett, 1937) is passed on—though in diminishing concentration—from cell to cell through successive divisions in sufficient amount to give the observed enhancement of developmental progression. Such an interpretation is consistent with the facts and has its analogy in Boysen Jensen's (1939) application of his 'Wuchstofftheorie' to geo-und plagiogeotropism.

Concentration of growth substances is not necessarily the sole factor. It is conceivable that mechanical pressure is contributory through induction of changes in form, tugor, permeability, and orientation of cells and cell contents. These, while not permanent, would affect the exercise of the preparatory processes taking place during its exhibition.

A while back we played with the idea that pressure—when present—might be a factor for differentiation. We were careful to point out that in our experiments the effect—if any—was not to be seen during centrifugation but only subsequent thereto. Now it is reasonable to suppose that orientation as well as chemical specialization of cellular contents is a *sine qua non* of differentiation. The very change in chemical as well as cytological structure ensures that this must be so. The progress

*There was a slight increase in stolon production in the A series which by itself is inconclusive but when taken with all the other data seems to justify its inclusion in the generalization.

of differentiation of muscle cells and tissue in the regenerating chela of the hermit crab permits an excellent visualization of the concurrent phenomena (Hammett and Hammett, 1932). It therefore follows that the orienting influence of directional application of centrifugal force may well have contributed to whatever cellular specialization was taking place in the preparatory changes of pedicular anlagen formation and reconstitution prior to the new hydranth production. While other mechanical effects may have participated, the foregoing is sufficient index of the potentiality.

The fact that subsequent expression of hydranth differentiation was not specifically forwarded is consistent with the interpretation. For, since centrifugation was not applied at any time during its exercise, but only during the exercise of the differentiation of initiation, it is obvious that pressure *per se* could have no special influence on the later occurrence of the activity. The only influence possible would be that resulting from growth substance accumulation as discussed in preceding paragraphs.

So far so good. But in the matter of stolon metaplasia we have something else. It was early found that tyrosine forwarded differentiation expression in obelia (Hammett, 1936a). It was demonstrated that tyrosine is specifically concentrated in the region of stolon metaplasia (Hammett and Chapman, 1938). It is therefore possible that induction of stolon metaplasia is related to tyrosine concentration in stolon cells or plasm. From this comes the possibility that centrifugation contributes to stolon metaplasia through concentrating tyrosine carriers or precursors from hydroplasm to stolon anlage. Orientation of stolon cells is as necessary to stolon metaplasia as is their chemical specialization. It is therefore possible that a pressure effect of centrifugation is also a contributor to metaplasia enhancement. Against this interpretation might be cited the fact that the hydranth which develops from a stolon is usually at the tip of a fairly long growth. The reply would be that hydranth appearance and development after its initiation is a very slow process as was determined by the cinematic record: while stolon growth in length is quite rapid. Therefore if, as was the case here, examination of stolons for metaplasia took place 22 hours after centrifugation, it could not have been otherwise than that the new hydranths should be found on a long stolon, and it could well have been that induction of hydranth initiation occurred at the beginnings of stolon growth.

The indifference of stolon length growth to centrifugation immediately after the hydrocaulus is cut is at present inexplicable on any other basis than its planotropic property as discussed earlier. Unless the idea be invoked that stolon formation from hydrocaulal endoderm was

delayed to such extent accumulation or deprivation of generally significant growth substances in the cells was insufficient to produce subsequent reaction after the first little influence on stolon production. This would not mean that the threshold value for the specific stimulus to metaplasia would not be passed: merely that the amount of general growth substances would not be sufficiently changed. It is also reasonable to assume that the specific determinant of stolon increase in substance may be different from that specific to hydrocaulus and hydranth increase in substance (Hammett, 1936). And it is possible that whereas the latter may be, like auxin, subject to geotropic influence, because its substrate is similarly subject: the former may not be influenced by gravity, because its substrate is essentially planotropic.

It may be thought too much attention has been paid to these speculative details. On the other hand it is only by examination of the possibilities and setting them forth that paths to solution are indicated. One function of scientific endeavor is to state these uncertainties and the possible paths to their solution.

SUMMARY AND CONCLUSION

In 23 experiments 454 obelia colonies were subjected to centrifugation for definite periods and the subsequent production and growth of hydranths and stolons recorded. It was found that the centrifugal push increased the number of hydranths produced from distal anlagen by natural growth; from cut distal ends of the colonies by regeneration; and from stolons by metaplasia. The subsequent developmental growth of these organisms was also increased. The oppositely acting or centripetal pull tended to hold back both production and growth. Stolon growth in length was indifferent to centrifugal force as used in these experiments. The implications of the findings are discussed in the text.

The work was done at The Marine Experimental Station of The Lankenau Hospital Research Institute, North Truro, Cape Cod, Massachusetts.

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SOME PHYSIOLOGICAL PHENOMENA FITTED TO GROWTH EQUATIONS

IV. TIME AND POWER RELATIONS FOR A HUMAN INFANT CLIMBING INCLINES OF VARIOUS SLOPES

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(*Received for publication February 28, 1940*)

INTRODUCTION

A fundamental behavior characteristic of infants observed by McGraw (1935) is their ability to climb inclined slides of various slopes. She has shown that a young child possesses this capacity shortly after learning to creep and prior to the attainment of erect locomotion. Specifically, *a child who is able to creep has the ability to climb inclined slides,* as may be seen by the accompanying photograph.

The present study was designed to ascertain whether the ability to ascend inclined slides is a growth phenomenon, that is, one which exhibits changes which can be interpreted in the light of existing growth equations. One of the purposes of scientific investigations is to discover principles or laws which will permit prediction of what will happen when the same conditions are again encountered. Like many physical phenomena, the causes of which are unknown or unexplained, growth is fundamentally a matter of change with advancing time. As with the physical phenomena an understanding of its nature will be furthered when the process has been described in physical language which uses physical concepts already established. Indeed, when this has been accomplished growth will rest on a basis comparable to that of any physical phenomenon which changes with time. The problem reverts to determining the amount of growth made in a given time measured in the proper units, the velocity of growth measured in growth units per unit time, and the acceleration of growth measured in growth units per unit time squared. Measurement of these quantities will enable us to state and define all the properties, effects, and relationships of the growth phenomenon in question without explaining, for the moment, its actual cause. Without knowing the cause of the changing ability of the infant to ascend slides of various heights we may measure the angle of the slide in degrees, the duration of the ascent of the slide in seconds, the weight and age of the child; with these measures we may seek to evaluate a governing law.

EXPERIMENTAL PROCEDURE

One child, James Briggs, was given daily tests five days a week on his ability to creep up a wooden slide which was clamped successively to stools of graded heights, thus forming inclines of varying angles. A space the same length as the slide (six feet) was marked out on the floor; this was considered to be an incline with zero angle. The following is a list of the slide angles used: 0°, 9.9°, 16.9°, 23.3°, 29.9°, 37.6°, 47.1°. The slide was sufficiently wide ($3\frac{1}{2}$ feet) so that the edges could not be grasped for support.

The experiment was started when the child was 237 days old. He was first induced by means of a lure to creep across the stretch on the floor. He was then placed at the bottom of the 9.9° angle slide and coaxed to creep up it. If he succeeded he was placed at the bottom of the 16.9° angle slide. The procedure was continued until he failed to make an ascent, whereupon the experiment was stopped for the day. He was not tried on the slides at a greater angle than the one on which he had failed. The times of ascent of the various slides on which he was

Photograph of a typical two year old child going up an incline of $58\frac{1}{2}$°.

successful were recorded by means of a stop-watch. His age and weight were also recorded each day.

Theoretical Derivation of Equation Relating the Time of Ascent of a Given Slide to the Age of the Child:—O. A. Simley (1933) in his monograph on learning says that there is no universal agreement as to what constitutes the fundamental process of learning, but that some structural change in nervous tissue is involved in learning seems to be unquestioned.

Let us assume that this "structural change in nervous tissue" constitutes a "growth process" and that this growth process is limited by the available resources necessary for growth. These resources, as Brody (1927) postulates, may be in the form of available food or energy, or available freedom from the encroaching by-products of growth, or there may be some growth-inhibiting force or substance in the environment which exerts a limiting influence; or as Wilson (1925) puts it, the limitation of growth is fundamentally determined by heredity and is the outcome of the specific organization of the germ-cells. Whatever may be the specific cause of the limitation of the growth process, we may assume (in order to evaluate this particular experiment) that the time of ascent of a given unit slide is a measure of the degree of learning or development which in turn is a measure of the "structural change in nervous tissue". Then if θ_m represents the limiting value of the time of ascent of a given unit slide, i. e., the minimum or fastest time in which the child was eventually able to ascend a slide one foot long, and θ represents the time of ascent of a given unit slide at any age, t, the difference between these two values, $(\theta\text{-}\theta_m)$ represents the concentration of limiting resources at any given age, t.

We may then postulate that the instantaneous velocity of change of time of ascent of a given unit slide with age is proportional to the value $(\theta\text{-}\theta_m)$ so that

$$1. \qquad \frac{d\theta}{dt} = k(\theta\text{-}\theta_m)$$

$(\theta\text{-}\theta_m)$ may then represent the potential improvement in proficiency yet to be made (or the degree of development of the phenomenon of ascending slides yet to be made) or as Brody (1927) puts it, $(\theta\text{-}\theta_m)$ may be called the "growth potentiality" or "growth impulse".

Equation 1. may be integrated.

$$\int \frac{d\theta}{\theta\text{-}\theta_m} = k\int dt$$

$$\ln\ (\theta\text{-}\theta_m) = kt + C$$

$$(\theta\text{-}\theta_m) = Be^{kt}$$

$$\theta = \theta_m + Be^{kt}$$

TABLE I

AGE, WEIGHT, AND TIME OF ASCENT FOR A HUMAN INFANT CLIMBING INCLINES OF VARIOUS SLOPES

		Time of Ascent in Seconds per Foot						
Age Days	Weight Pounds	Incline 0°	Incline 9.9°	Incline 16.9°	Incline 23.3°	Incline 29.9°	Incline 37.6°	Incline 47.1°
240	19.9	7.00	12.18	12.58	16.10	——	——	——
250	20.0	3.25	8.03	7.38	4.92	5.93	10.30	——
260	20.3	1.52	3.92	3.82	5.05	6.50	6.27	——
270	20.6	1.13	2.32	2.58	3.45	4.80	6.60	——
280	20.9	0.88	2.18	1.92	3.00	3.60	6.27	17.58
290	20.8	0.85	2.47	2.38	2.98	3.58	9.72	8.62
300	21.0	0.90	1.83	2.08	2.65	2.88	4.90	10.17
310	21.3	0.95	1.47	1.78	1.92	2.73	4.43	14.12
320	21.4	0.72	1.50	1.75	2.20	2.47	4.17	11.08
330	21.8	0.65	1.13	1.45	1.62	2.17	3.47	8.58
340	22.3	0.57	1.12	1.10	1.40	1.97	4.60	12.73
350	22.2	0.62	1.03	1.20	1.52	2.47	4.80	9.23
360	22.6	0.58	0.95	1.07	1.15	1.82	2.33	4.38
370	22.9	0.83	0.92	1.27	1.55	1.85	3.28	6.03
380	22.8	0.72	0.97	1.00	1.12	1.63	2.33	4.57
390	23.3	0.65	0.78	1.13	1.28	1.92	2.73	——
400	22.7	0.78	0.93	0.98	1.13	1.55	2.12	3.72
410	23.3	0.65	0.75	0.92	1.10	1.57	2.03	2.90
420	23.3	0.67	1.25	1.02	1.17	1.05	2.52	2.87
430	23.5	0.75	0.83	0.90	1.20	1.32	2.42	3.82
440	23.5	0.73	0.87	0.82	1.37	1.42	2.53	2.68
450	23.9	0.92	1.08	1.05	1.20	1.60	2.37	3.08
460	24.0	0.97	1.10	1.83	1.60	2.25	3.30	5.33
470	23.4	0.82	1.52	2.55	1.90	2.45	2.87	4.55
480	23.5	0.72	1.32	1.80	1.38	2.12	2.93	5.47
490	23.9	0.80	1.13	1.27	1.77	1.88	3.10	5.18
500	24.6	1.02	1.18	0.95	1.18	1.70	2.55	3.93
510	24.5	0.87	1.02	1.02	1.12	1.95	2.63	3.70
520	24.5	0.65	0.88	1.02	1.22	1.37	2.00	3.80
530	25.0	0.60	0.83	1.00	1.15	1.53	2.02	3.23
540	25.2	0.72	1.23	1.18	1.55	1.72	3.00	3.65
550	25.3	0.68	1.50	1.47	1.60	1.62	2.35	3.25
573	24.5	0.85	0.97	1.72	1.25	1.42	2.10	2.25
580	25.1	0.82	0.97	1.32	1.67	1.98	2.67	3.77
590	25.6	0.87	0.68	0.73	1.18	1.87	2.08	3.27
600	25.6	0.60	0.72	1.13	1.05	1.57	2.03	2.38
610	25.4	0.65	1.03	1.02	1.13	1.28	1.53	2.22
620	25.7	0.60	0.78	0.78	0.82	0.98	1.73	1.82
655	26.1	0.52	0.73	0.85	1.10	1.17	1.48	1.85
715	27.3	0.57	0.83	0.70	0.53	0.70	1.07	1.82
730	27.5	0.53	0.72	0.63	0.58	0.82	1.12	1.93
740	27.5	0.50	0.53	0.67	0.52	0.57	0.87	1.42

2. $$\theta = \theta_m + \theta_m e^{k(t-t')}$$

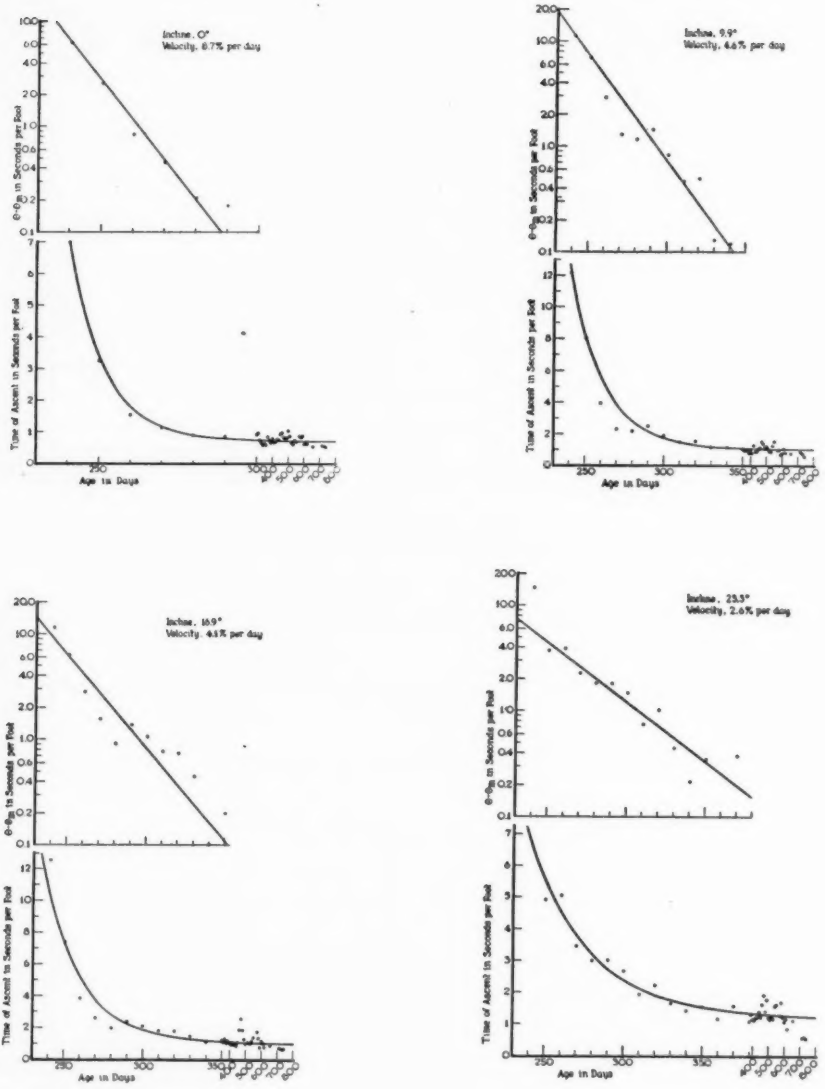
which gives the equation relating the time of ascent of a given unit slide, θ, to the age of the child, t. In equation 2. the value of k gives the time-rate of change in the time of ascent of the given unit slide, and 100k gives the percentage rate of change; t′ is a constant, and is the age when the time of ascent is twice the minimum time. When $t = t'$, $\theta = 2\theta_m$. e is the base of natural logarithms, 2.718. Equation 2. is the same form as that fitted to the change in duration of the Moro reflex with age (Weinbach, 1938), the growth of brain potentials (alpha frequency) in the human (Weinbach, 1938b) and the growth of various animals, plants, bacteria, cells, and populations (Brody, 1927).

Experimental Results:—Table I gives the times of ascent of the various slides in seconds per foot at ten day intervals. Each value represents an average of usually seven determinations as the child was not observed on Saturdays and Sundays. Table I also gives the weight of the child in pounds.

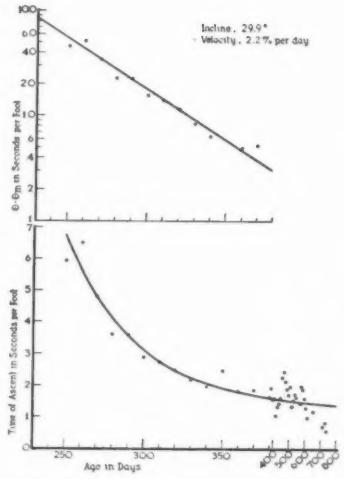
Fit of Equation 2. to the Experimental Data:—The data are fitted to this equation by determining graphically the asymptote, or minimum time of ascent, θ_m and plotting $(\theta\text{-}\theta_m)$ on semilog paper versus the age of the child in days. A straight line should result as shown in the upper part of Figs. 1, 2, 3, 4, 5, 6, and 7, since $\ln(\theta\text{-}\theta_m) = kt + \ln B$ is linear in $\ln(\theta\text{-}\theta_m)$ and t. The velocity constant, k, is evaluated as the slope of the semilog plot and t′ is read from the graph as that age when the time of ascent is twice the minimum time. If θ_m is chosen too high, the graph will curve downward, and if θ_m is chosen too low, the graph will curve upward.

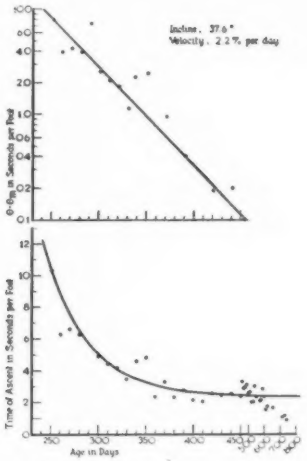
In the lower half of Figs. 1, 2, 3, 4, 5, 6, and 7 the data from Table I for the various inclines are plotted. The ordinates give the times of ascent of the various slides in seconds per foot and the abscissae give the age of the child in days. The smooth curves are theoretical curves of equation 2. as obtained from the semilog plots shown in the upper part of the figures. Table II gives a summary of the constants obtained from these theoretical curves. Note that in general the steeper the slide the slower the rate of development in the ability to ascend it. The column marked k_0/k in Table II is the ratio of the velocity constant for creeping on a flat surface to the velocity constants for the various slides. The value of k_0/k indicates the number of days required to accomplish a development for each slide equivalent to one day of development of creeping on a flat surface.

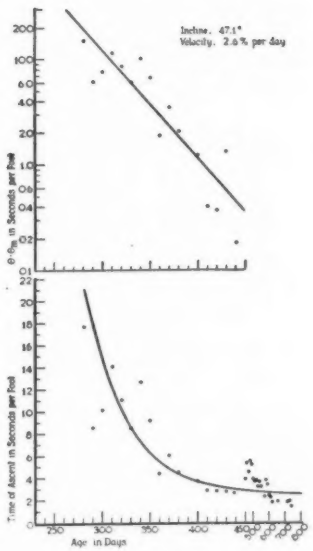
Relation Between Power Output in Climbing the Slides and the Age of the Child:—Since power is the rate of doing work it seemed of interest to determine how the power output changed as the child's ability to



Figs. 1, 2, 3, and 4. Chart of data given in Table I. The lower part of the chart is a plot of the time of ascent of the given incline in seconds per foot as ordinates versus the age of the child in days as abscissae. Upper part of chart gives the time of ascent of the given incline minus the minimum time as ordinates on semilog paper versus the age in days as abscissae.







Figs. 5, 6, and 7. Chart of data given in Table I. The lower part of the chart is a plot of the time of ascent of the given incline in seconds per foot as ordinates versus the age of the child in days as abscissae. Upper part of chart gives the time of ascent of the given incline minus the minimum time as ordinates on semilog paper versus the age in days as abscissae.

TABLE II

SUMMARY OF CONSTANTS OBTAINED FROM THE CHARTS OF TIME OF ASCENT VERSUS AGE

Incline	θ_m sec. per foot	k per day	t' days	k_0/k ratio
0°	0.67	.087	266	1.0
9.9°	1.00	.046	289	1.9
16.9°	1.00	.041	295	1.7
23.3°	1.17	.026	301	3.3
29.9°	1.33	.022	313	3.9
37.6°	2.33	.022	307	4.0
47.1°	2.50	.026	367	3.3

θ_m is the minimum time of ascent; k is the velocity constant of growth; t' is the age when the time of ascent is twice the minimum time; and k_0/k indicates the number of days required to accomplish a development for each incline equivalent to one day of development of creeping on a flat surface.

climb the various slides improved. Power is defined by the relation

3. $$P = \frac{Wh}{\theta}$$

where P is power in foot-pounds per second, W is the weight of the child in pounds, h is the height of a unit slide, that is, a slide whose length is one foot, and θ is the time of ascent of the given unit slide in seconds. Equation 3. may be written

4. $$P = \frac{W\sin\alpha}{\theta}$$

sinse $h = \sin\alpha$, where α is the angle of the slide in degrees. But the time of ascent of the slide, θ is given by equation 1. so that

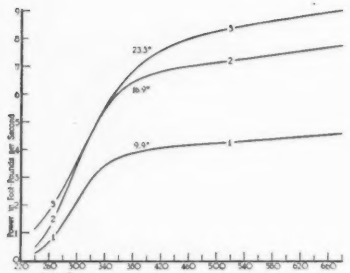
5. $$P = \frac{W\sin\alpha}{\theta_m - \theta_m e^{k(t-t')}}$$

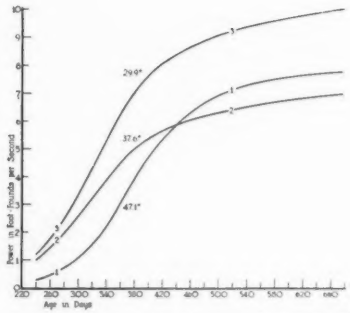
Equation 5. gives the theoretical relation between power output and age for ascending inclines of various slopes. The power output was calculated from equation 5. using the weights at various ages. Table III gives the power output for the angles of the inclines at different ages, and Figs. 8 and 9 are the graphs of power against age. From these graphs it is seen that there is a maximum power approached for each incline, and that for the low and high angles the maximum power approached is less than for some intermediary angle. This is brought out in Fig. 10 in which the power in foot-pounds per second is plotted against the load in per cent of the body weight, i. e., the fraction of the body weight carried up the incline. The load carried is $W\sin\alpha$, therefore the fraction of the body weight carried up the incline is given by $\sin\alpha$. Notice that there is an *optimum load as a fraction of the body weight for maximum power output.* This optimum load was approxi-

TABLE III

AGE, SMOOTHED WEIGHT, AND POWER OUTPUT FOR A HUMAN INFANT CLIMBING INCLINES OF VARIOUS SLOPES. THE POWER OUTPUT WAS CALCULATED FROM THE SMOOTH CURVES OF TIME OF ASCENT VERSUS AGE

		Power Output in Foot-Pounds per Second						
Age Days	Smoothed Weight Pounds	Incline 0°	Incline 9.9°	Incline 16.9°	Incline 23.3°	Incline 29.9°	Incline 37.6°	Incline 47.1°
240	19.9		0.28	0.56	1.16	1.23	0.99	0.28
250	20.0		0.43	0.81	1.43	1.48	1.18	0.35
260	20.3		0.63	1.16	1.78	1.80	1.42	0.44
270	20.6		0.93	1.60	2.16	2.14	1.68	0.56
280	20.8		1.29	2.08	2.60	2.53	1.95	0.69
290	21.0		1.69	2.76	3.06	2.95	2.25	0.87
300	21.2		2.14	3.42	3.55	3.40	2.56	1.06
310	21.4		2.54	4.04	4.05	3.89	2.89	1.30
320	21.7		2.92	4.68	4.56	4.40	3.16	1.58
330	21.9		3.28	5.01	5.04	4.90	3.56	1.89
340	22.1		3.42	5.54	5.50	5.38	3.88	2.25
350	22.3		3.58	5.89	5.88	5.82	4.18	2.63
360	22.4		3.70	6.09	6.25	6.24	4.44	3.02
370	22.6		3.77	6.26	6.53	6.64	4.71	3.45
380	22.8		3.84	6.44	6.84	7.02	4.94	3.86
390	23.0		3.92	6.56	7.06	7.35	5.15	4.26
400	23.1		3.94	6.65	7.25	7.58	5.32	4.64
410	23.3		4.01	6.71	7.44	7.85	5.50	5.02
420	23.4		4.02	6.74	7.59	8.05	5.62	5.34
430	23.5		4.04	6.84	7.69	8.20	5.74	5.63
440	23.7		4.07	6.89	7.81	8.39	5.89	5.91
450	23.8		4.09	6.93	7.91	8.54	5.95	6.12
460	23.9		4.11	6.95	7.95	8.64	6.04	6.30
470	24.1		4.14	7.01	8.08	8.77	6.13	6.45
480	24.2		4.16	7.04	8.11	8.86	6.19	6.64
490	24.3		4.18	7.07	8.15	8.97	6.24	6.77
500	24.5		4.21	7.12	8.21	9.05	6.31	6.89
510	24.6		4.24	7.15	8.31	9.09	6.36	6.99
520	24.7		4.25	7.19	8.35	9.19	6.41	7.05
530	24.8		4.26	7.22	8.38	9.24	6.45	7.14
540	24.9		4.28	7.24	8.41	9.26	6.48	7.19
550	25.0		4.30	7.27	8.45	9.30	6.50	7.25
560	25.1		4.32	7.30	8.48	9.34	6.55	7.30
570	25.3		4.35	7.35	8.54	9.49	6.60	7.36
580	25.4		4.37	7.39	8.59	9.52	6.62	7.39
590	25.5		4.39	7.42	8.61	9.56	6.65	7.45
600	25.6		4.40	7.45	8.65	9.60	6.68	7.47
610	25.7		4.42	7.48	8.69	9.64	6.74	7.50
620	25.9		4.45	7.54	8.74	9.71	6.79	7.56
630	26.0		4.47	7.56	8.78	9.75	6.81	7.59
640	26.1		4.49	7.59	8.81	9.79	6.84	7.65
650	26.3		4.52	7.65	8.88	9.86	6.89	7.71
660	26.4		4.54	7.68	8.92	9.90	6.91	7.74
670	26.6		4.57	7.74	8.99	9.97	6.96	7.80
680	26.7		4.59	7.76	9.02	10.01	6.99	7.83





Figs. 8 and 9. Chart of data given in Table III. Power output as ordinates (calculated from the smooth curves of time of ascent versus age) versus the age of the child in days as abscissae.

mately 40 per cent of the child's body weight at all ages. This corresponds to an *optimum slide angle for maximum power output of about* 24.0 *degrees.* Thus there seems to be an optimum load or resistance to bring forth the maximum power output in the child. Further, the peak performance at the optimum angle becomes increasingly greater as the child gets older.

These power curves probably also give in general the picture of the energetic efficiency in climbing the various inclines, since the energetic efficiency increases with increasing rate of work (Brody and Trowbridge, 1937). The curves of Fig. 10 for power output versus the sine of the angle of the incline are interpreted as the relation between the power output and load. Viewed in this manner the curves of Fig. 10

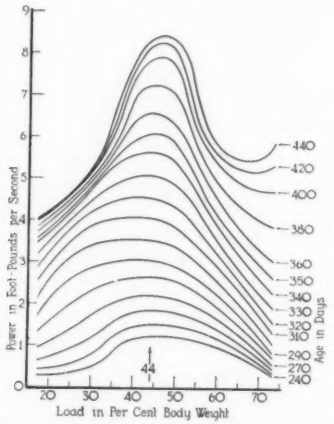


Fig. 10. Chart of the power output as ordinates against the load in per cent of the body weight carried up the incline, as abscissae for various ages.

are similar to the curves of energetic efficiency versus speed in climbing steps for a human adult, the energetic efficiency versus the load for horses, and the gasoline consumed versus the speed for an automobile, as given by Brody and Trowbridge (1937). Obviously, when the load is zero, no work is accomplished, and when the load is too great to be overcome, no work is accomplished. Somewhere between these two points there is an optimum load for maximum power output and efficiency.

SUMMARY

The fundamental problem of describing various aspects of growth in terms of physical equations which express change with advancing time has been extended by a study of the behavior of one child during the development of proficiency in ascending inclines of various slopes.

The times of ascent of the various inclines were plotted against age and the data fitted to a theoretically derived growth equation from which the velocity of growth in the ascent of the inclines could be evaluated.

The power output in climbing the inclines was determined and the curves of power output versus age were plotted. As the child grew older, the power output rose and approached a maximum for each incline. When power output was plotted against the fraction of the body weight carried up the incline, i. e., the load, it was noted that there was an optimum load of approximately 40 per cent of the body weight to bring forth a maximum power output. This corresponds to an optimum angle of about 24.0 degrees for maximum power output. The optimum load or optimum angle remained essentially unchanged with advancing age.

ACKNOWLEDGMENT

I am indebted to Vera Dammann for making this paper possible by her diligence in carrying the experimental work through to completion; to Myrtle B. McGraw for her kind help and suggestions; to Robert S. Childs for able assistance in preparing the charts and manuscript; and to Kenneth W. Breeze for his careful lettering and drawing of the charts.

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THE ACTION OF GROWTH STIMULANTS ON PROTEINS*

By

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(Received for publication March 2, 1940)

A series of complicated chemical reactions undoubtedly occurs during tissue growth. This study deals with the chemical effects of reputed growth stimulants on proteins in vitro as related to the sulphydryl grouping.

Tissue proliferation stimulants are of interest in the field of malignant growth and in normal physiological repair. Proliferation is stimulated in the development of cancer (Loeb et. al., 1937; Hammett, 1935; Owen, 1937, and others).

Among the reported growth stimulants are the sulphydryl containing complexes (Hammett, 1929) blow fly larva (Baer, 1931) larval preparations (Livingston, 1932) allantoin (Greenbaum, 1936) urea (Hetherington et. al., 1937) calcium salts (Stewart, 1934) and dextrose (Lafresniere, 1936). In addition embryo extracts have long been used in tissue culture media. Of the above, free sulphydryl groups may readily be demonstrated in the sulphydryl complexes, in appropriate larval preparations and in the freshly prepared embryo extracts. Non-antigenic extracts of embryonic tissues may be made which are effective in stimulating growth (Owen and Cutler, unpublished) and a discussion of the probable chemical mechanisms of growth stimulation has been presented by Owen (1938).

PROCEDURE

The release of free sulphydryl by growth stimulants and carcinogenics from proteins in vitro was tested for as follows: Saturated solutions of the chemical growth stimulants were made up in phosphate buffers at pH 5.8 and 7.4. One per cent solutions of edestin and of crystalline egg albumin were prepared in similar buffers. Pooled sera were also utilized for a protein agent. The positive control solution for purposes of sulphydryl testing was a 1 to 100,000 cysteine hydrochloride solution freshly made. Appropriate reagent and solvent controls were also run. The phosphotungstate procedure as used by Owen (1938) was employed to determine the presence of the sulphydryl group. Cystine but not cysteine was demonstrable in the control proteins and in the serum.

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For the test, equal volumes of the growth stimulating solution and the protein solution or diluted serum were mixed. A portion (4 cc) was tested immediately and a second portion incubated at 37.5 C for two hours then tested for the —SH group.

The influence of the carcinogenics on proteins was tested in a similar manner except that the solid carcinogens were added to the protein solutions previous to testing or incubation.

RESULTS

Testable sulphydryl appeared in the albumin-urea and in the edestin-urea mixtures. Other growth stimulants and the carcinogenics failed to cause the appearance of free —SH on mixture with proteins in either acid or alkaline buffer solutions, nor was a positive test caused to appear by two hours incubation. Electrometric determinations indicated that allantoin did release some free —SH but this was not demonstrated chemically. Calcium salts, dextrose and the carcinogenics methylcholanthrene, dibenzanthracene and benzpyrene failed to induce any release of free sulphydryl by either chemical or electrometric tests. Less than saturated solutions of urea with proteins gave less positive sulphydryl tests. The stronger test was obtained when the urea-albumin mixture was held at a pH of 7.4, and similar findings were noted for the urea-edestin mixtures. Incubation of the protein mixtures alone failed to show release of the free —SH group. Serum proteins altho containing cystine did not release testable sulphydryl when in contact with the growth stimulants. This may have been a matter of concentration of the proteins.

DISCUSSION

Substances which directly or indirectly release sulphydryl groups from proteins should act as growth stimulants. Greenstein (1939) used urea and guanidine hydrochloride as solvents for proteins and obtained sulphydryl release. It may be said that the higher melting points of these solvents were not alone the cause of the appearance of free sulphydryl groups. In addition it may be stated that the substances which release urea by mild heat or by hydrolysis would be expected to cause growth stimulation. Allantoin in a solution in the presence of heat or alkali hydrolizes into urea and either allanturic acid or glyoxylic acid. The urea thus released may then act on wound proteins as stated above. The fact that allantoin is fairly insoluble may explain why we failed to detect chemically the release of free sulphydryl from proteins by its use although electrometric results indicated such a release. The concentration of sulphydryl essential to stimulate growth or tissue proliferation is extremely small and would not be picked up by the usual available chemical tests (Hammett, 1938). Substances which tend to produce a reducing or alkaline condition in wounds might also

be expected to cause tissue proliferation, as the reduction of sulphur-sulphur linkages in tissue proteins would be favored thus producing the essential free sulphydryl complex.

Means other than chemical will be utilized to detect the minute concentrations of the sulphydryl grouping essential to stimulate tissue proliferation and results of these tests will be given at a later date.

SUMMARY

1. It has been shown that certain growth stimulants as urea release testable sulphydryl on contact with proteins.

2. Since sulphydryl compounds stimulate tissue proliferation, non-sulphydryl complexes which cause sulphydryl to be released on contact with proteins may serve indirectly as growth stimulants.

3. Substances which release urea on contact with body tissues may be expected to stimulate tissue growth rates; among these may be enzymes as arginase.

The author wishes to express appreciation to Dr. M. Cutler for his assistance in preparation of this report.

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THE GROWTH REACTION OF OBELIA GENICULATA TO D(-)-THREONINE

By

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(Received for publication March 26, 1940)

The discovery and isolation as a cleavage product of proteins of a new amino acid by McCoy, Meyer, and Rose (1935; 1936) its identification as alpha-amino-beta-hydroxy-*n*-butyric acid; and their demonstration that the compound is an indispensable component of the diet, made desirable an exploration of the possibility the substance may have some specific role to play in developmental growth.

For this purpose the colonial-living hydroid *Obelia geniculata* was used because it has so far been found to be the organism best suited for investigations of this nature. A recent report carries the key references (Burr and Hammett, 1939). The d(-)-threonine was generously supplied by Prof. Wm. C. Rose who prepared, isolated, purified, and identified the compound for our use. The usual experimental procedure was followed. Stock sea-water solutions were made daily or more often when required. From these, aliquots were added to test culture solutions in amounts adequate to give M/400,000; M/200,000; and M/100,000 concentrations. Test and control-cultures were run simultaneously under like conditions of temperature, illumination, and pH. Four changes into fresh solutions were made during the 24-hour course of experiment. There were 30 experiments with close to 16,000 animals, half of which were exposed to d(-)-threonine in the stated concentrations. The results are given in Table I which is derived, constructed, and analyzed as described elsewhere (Hammett and Schlumberger, 1937).

Full credit for exemplary technical assistance throughout should be given to Misses Steele and Hyde and Messers Toman and Rivard.

RESULTS

D(-)-threonine seemed toxic to obelia growth at M/100,000. Thus—whereas at M/200,000 test growth was greater than control in 2 of the 4 developmental activities and less in 1, at M/100,000 it was less in 2 and greater in 1. Further, of the 34 possible growth expressions at M/200,000, 15 were greater in test than control and 11 were less, while at M/100,000, 13 were less and but 9 were greater. The shifts towards decrease in expression of catabolic disintegration, and increase in regression activity which occurred at M/100,000 in contrast to the re-

*Aided by a grant from the International Cancer Research Foundation.

TABLE I

TREND OF TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENTAL AND METABOLIC ACTIVITY IN d(-)- THEONINE CULTURES. ALSO THE NUMBER OF EXPERIMENTS, COLONIES, HYDRANTHS, AND GONOPHORES AT THE STATED CONCENTRATIONS.

M/1000x	400			200			100		
No. Expts.	10			10			10		
	Cont.	Test		Cont.	Test		Cont.	Test	
Colonies	149	149		150	150		147	147	
Hydranths	2599	2600		2780	2776		2654	2664	
Gonophores	379	400		377	313		281	278	
	GROWTH								
	+	—	=	+	—	=	+	—	=
Initiation	3	3	1	1	5	1	1	4	2
Proliferation	4	3	1	7	1	0	4	2	2
Differentiation	3	3	4	5	2	3	2	4	4
Organization	3	1	4	2	3	4	2	3	3
	METABOLISM								
	By Single Experiment								
Maintenance	5	3	2	4	4	2	4	5	1
Catabolism	6	4	0	9	1	0	4	5	1
	By Combined Experiment								
%	Cont.	Test		Cont.	Test		Cont.	Test	
Regression	20.9	21.2		24.6	24.7		28.9	29.1	
Catabolism	76.4	77.9		78.8	86.5		87.6	86.4	
	Increase In Percentage Regression								
%	1.4			0.4			0.7		

actions at M/200,000 and M/400,000 are consistent with the inference.

At M/400,000 there was no sure indication of any growth influence which carried over into the higher non-toxic M/200,000 concentration. Therefore the reaction in M/200,000 cultures will be taken as the starting point for analysis.

Initiation was retarded in the presence of d(-)-threonine. The evidence is: test was less than control in 29 and greater in but 16 of the 70 observations, and less in 5 and greater in but 1 of the 10 experiments done at this concentration. Further, it was less in 5 and greater in but 1 of the 7 common expressions of this growth activity.

Proliferation expression was definitely enhanced. Test was greater than control in 24 and less in but 8 of the 80 observations, and greater in 8 and less in but 1 of the 10 experiments. Further, it was greater in 7 and less in but 1 of the 8 potentialities for activity expression. This reaction trend was hinted at in the M/400,000 experiments and persisted in the M/100,000 somewhat toxic range where other developmental growth activities were retarded.

Differentiation also was enhanced. Thus—test was greater than

control in 26 and less in but 16 of the 100 observations and greater in 5 and less in but 2 of the 10 experiments. Further, it was greater in 5 and less in but 2 of the 10 potentialities for activity expression. No hint of such influence was shown at M/400,000, and retardation rather than advancement occurred at M/100,000.

Organization expression was essentially unaffected by d(-)-threonine. Thus—test was less than control in 12 and greater in 16 of the 90 observations, and greater in 3 and less in 2 of the 10 experiments. On the other hand 3 of the 9 expressions of this growth activity were less in test than control, while 2 were greater.

Maintenance and regression of complete hydranths was unaffected at any concentration. There was no evidence of specific dynamic effect. Catabolic disintegration of the senile animals was stimulated in the non-toxic M/400,000 and M/200,000 concentrations.

DISCUSSION

The apparent toxicity at M/100,000 is unique: no other amino acid showing a like effect at this low concentration.

The absence of regression effect and specific dynamic action expression despite the added CH_2 (Hammett and Toman, 1939) may be attributed to the dampening effect on these processes of the OH group (Hammett and Rivard, 1938). The results are at least not inconsistent with these previous interpretations.

The retardation of initiation expression is not an uncommon finding with amino acids. Its basis must await more detailed correlative analysis.

It is possible the increase in differentiation expression is secondary to proliferation enhancement. Such has been shown to occur (Hammett, 1931). Evidence in this direction is threefold, viz. no hint of reaction occurs at M/400,000 where suggestion of proliferation step-up is present: the reaction is not sustained in the toxic range as is that of proliferation; and the extent of reaction at M/200,000 is consistently less than cell increase in number.

The outstanding response to d(-)-threonine is the enhancement of proliferation expression. This is sufficiently marked in degree and consistent in occurrence to make the event beyond cavil.

In order to rule out any possibility that this result might have been due to contamination of the compound with SH or SH-precursors as was found to be the case with natural l-leucine (Hammett, 1940) we asked Dr. Jesse P. Greenstein of the National Institute of Health to examine the sample for sulfur with the exquisitely delicate methods he has devised. Dr. Greenstein kindly undertook this task and reported back that the sample of d-threonine we submitted as an aliquot of that

used in these experiments showed no trace of sulfur in any form.

This being so it appears as if we have in this compound another group of specific significance to cell increase in number. Interesting enough is the fact that the introduction of an OH group into the amino acids or nucleic acid derivatives seems to determine in most cases a propensity to forward expression of this growth activity. Thus hydroxy-proline has such action while proline does not, (Hammett, 1937; Hammett and Collings, 1937); serine exerts some effect while none is shown by alanine (Hammett and Rivard, 1938; Hammett, 1936): thymine is more active in this direction than cytosine (Hammett and Lavine, 1937; Hammett, Lavine, and Lavine, 1937); and xanthine than hypoxanthine also (Hammett and Porter, 1938; Hammett and Steele, 1938): guanine is favorable while adenine is not (Hammett, 1938; Hammett, 1938a). Uracil may or may not be an exception as contrasted with cytosine (Hammett, 1936a; Hammett, Lavine and Lavine, 1937): but tyrosine is surely an exception when compared with phenylalanine (Hammett, 1936).

These associations open up the possibility that the OH group like the SH is of particular significance to some phase of the proliferation process. On the other hand they suggest that since both have similar redox properties they may both be of influence in the same process. Not to be forgotten however is the possibility of interchange of SH for OH in the activities of living; perhaps that OH may in some circumstances be a precursor of SH. For this latter idea there is some evidence and speculation.

Whatever the relation may be, the results present a thread of connection which suggests and opens a new horizon for exploration.

The growth promoting property of threonine exhibited here substantiates similar findings elsewhere (Maeda, Higasi, and Matuoka, 1938).

SUMMARY AND CONCLUSION

Thirty experiments with close to 16,000 animals (*Obelia geniculata*) half of which we exposed to d(-)-threonine in M/400,000; M/200,000; and M/100,000 concentrations showed that the amino-acid has a definite forwarding action on the proliferation phase of obelia development, with a similar but less marked and probably secondary effect on differentiation.

Initiation was held back and organization was unaffected as were the metabolic activities of maintenance and regression. Catabolism was increased.

The work was done at The Marine Experimental Station of The Lankenau Hospital Research Institute, North Truro, Massachusetts. with generous aid from Miss A. E. Bein of Philadelphia.

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THE EFFECT OF FLUORESCENT DYES ON THE GROWTH OF PLANTS

By

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(*Received for publication April 29, 1940*)

In 1925, Boas together with Merkenschläger showed that the dyestuff Eosin, even in as weak solutions as 1:640,000, completely inhibits the geotropic reaction of the germ roots of wheat. According to Boas' opinion, Eosin—by changing the stimulation, conduction and distribution of growth-promoting substances—seems to change the direction of the growing root and plant. In addition to these deviations of the roots after Eosin treatment, which is of a passive character, there also are active curvatures caused by negative chemotropism.

The plant experiments with dyes herein described were carried out from quite a different point of view. They originated from the standpoint that, since it is possible to render men and animals more sensitive to the sun's rays by means of dyes, the same ought also to apply to plants. To this whole problem-complexum it appeared of utmost importance that the Chlorophyll—which is a fluorescent dye itself—was considered as a *light catalyzer* by many authors. So the plant is, ipso facto, "disposed" to be rendered still more sensitive to light with dyes. Another consideration also gave impulse to investigation of this question: this was the close chemical relation between the Chlorophyll and the blood dye "Haemoglobin". (See publications about Porphyrins, Chlorophyll-Porphyrins, Heamatoporphyrins (Willstätter)). Commentaries concerning the Chlorophylls' chemical constitution and physical behavior—(fluorescence) on the one hand, and of the bile dyes, the Haematoporphyrin, which is also a fluorescent dye, on the other, showed that fluorescent dyes have an important influence on human, animal and plant organism also. This relationship between animal and plant organism was the scientific basis which led to investigation of the effect of fluorescent dyes on plants. The effect of fluorescent dyes on animals and human beings is already well known, as the following examples show: experiments from Tappeiner and Jodlbauer showed that animals can be sensitized to the sun. White mice—exposed to the sun after a subcutaneous injection of a few drops of a highly diluted Eosin solution—become restless, scurry and jump around in their cage, and die after some hours. If taken into a dark room before they are exhausted, they will slowly recover. Gray mice—not depigmented—cannot be sensitized to the sun with Eosin in the same way. Sheep fed with buckwheat are highly sensitized to sunlight and subsequently soon die off. Buckwheat contains the chemical material which causes the

light sensitizing (Fagopyrism). There are many other foodstuffs known to sensitize animals to sunlight.

Acridin dyes render human beings and animals very sensitive to ultraviolet light and sunlight. Some people are very sensitive to sunlight because their systems contain a great quantity of Porphyrin.

The photographic film and plate which contains a light sensitive substance (silver bromide) can be made more sensitive to light with certain dyes, especially light to which it was not sensitive at all (orthochromatic films to red and yellow, panchromatic films also to green light). This kind of sensitization is a photo-chemical process.

It is possible that the sensitizer reacts chemically with the sensitive material itself, but the sensitizer usually remains unchanged. According to the Quantum Theory, the sensitizer absorbs the energy quantum of the incident radiation and transfers this energy to the molecule which subsequently falls to pieces. The sensitizer regains its original form ready to absorb new energy quants.

The analogy between the above-mentioned process and the plant is evident. Here the Chlorophyll is the light-sensitive substance which can be rendered more sensitive by means of fluorescent dyes—for instance: Fluorescein. In the plant it is not the Chlorophyll alone which is sensitive to light, but the entire plant above the ground (Phototropism). This property of the whole plant can be raised by fluorescent dyes.

It was possible to prove with various especially fluorescent dyes that when highly diluted (1:100,000-1:2,000,000) they have an accelerating effect on plant growth. When applied in strong concentrations (½%-3%) they retard development. The fact has been verified in many hundreds of experiments, in Great Britian, Germany, Yuma (Arizona) U. S. A., and many other places.

EXPERIMENTS

In the earlier experiments, the fluorescent dyes were used alone in a concentration from 1:100,000-1:2,000,000; later, after having found that the effect of dyes can be raised by the catalytic action of iron and copper salts, small amounts of iron and copper sulfate were added to the fluorescent dyes—which, in most cases, was Fluorescein. In this mixture, which was named *PhotoSensin* and used always in a diluted form, the concentration of the dyes still remained 1:100,000-1:2,000,000. The following experiments were carried out mostly with PhotoSensin which, besides the acting materials, contains enough of a filling material to render the handling of it easier. The solutions used were prepared by dissolving 1 gram of PhotoSensin powder in 10-175 liters of plain water. The exact solution used will be mentioned when describing the experiments.

A. *Experiments with Beans*:

(1) *Hungarian Yellow Beans*:

(Municipal Gardeners' Training School, Budapest, Hungary.)

A test experiment was carried out with potted beans, making two series each containing 10 pots. Each pot received 500 grams of a well fertilized soil.

Series (a) was treated with PhotoSensin.

Series (b) was untreated, and received only plain water, and was the control series.

The PhotoSensin solution for series (a) was prepared by dissolving 1 gram of PhotoSensin powder in 24 liters of plain water (about 6 galls.). Into each pot was poured 100 cm^3 of this solution at each treatment.

Series (b) at the same time had to receive as much plain water as PhotoSensin solution was added to series (a).

All the pots of both series were placed in the warm open ground where they got not too much sun. Series (a) received a PhotoSensin treatment every 8-10 days. On days between treatments all the pots had plenty of water. It was evident that the PhotoSensin-treated plants

Fig. 1. *Beans* untreated.

Fig. 2. *Beans* treated with PhotoSensin.

were doing slightly better as early as the third week. Stems and leaves were a little larger than those of the controls. Later the leaves became still sturdier and the growth of the whole plant was more active. By the 6th-7th week young blossoms were already observed which steadily increased in number. The treated plants were not only sturdier, but there were more pods. The pods also were slightly larger.

In the experiments which were carried out, as mentioned above, the beans were treated with PhotoSensin nine times.

The average number of pods *in one pot* was:

(a) PhotoSensin-treated pots	36
(b) Untreated	12

That means an increase of over 100%.

The average length of the pods was:

(a) PhotoSensin-treated pods	10½ cms.
(b) Untreated	7½ cms.

Average size of leaves:

(a) PhotoSensin-treated plants	8 × 10 cms.
(b) Untreated plants	4½ × 7½ cms.

(2) *"Non Pareil" Beans*:
(Municipal Gardeners' Training School, Budapest, Hungary.)

This experiment was also carried out in pots, but the dye solution used was different in three series each containing 10 pots, each pot containing 500 grams of a well fertilized soil. The beans were all sown July 15, 1937 and harvested October 15, 1937.

Series	Treated with	Average No. stems in each pot	Average No. pods in each pot
Control	Plain Water	2	2
No. 1	Solution (1)	2	9
No. 2	Solution (2)	2	13
No. 3	Solution (3)	2	20

1st treatment, July 23, 1937, 100 gram solution per pot
2nd treatment, Aug. 7, 1937, " " " " "
3rd treatment. Aug. 22, 1937, " " " " "
Solution (1): 0.487 gr. Fluorescein; 0.080 gr. Iron Sulphate; 0.0060 gr. Copper Sulphate, dissolved in 100 liters water.
Solution (2): 0.286 gr. Fluorescein; 0.0457 gr. Iron Sulphate; 0.0034 gr. Copper Sulphate, dissolved in 100 liters water.
Solution (3): 0.203 gr. Fluorescein: 0.0320 gr. Iron Sulphate; 0.0024 gr. Copper Sulphate, dissolved in 100 liters water.
To each of these solutions sodium-bicarbonate had been added to make the dye water soluble.

This experiment shows conclusively the action of dyes on plants. All three treated series resulted in considerably more pods than the un-

treated control plants. It can also be seen that there is a difference in the action of the various dye concentrations. In the above case, the more diluted solution proved better than the less diluted one, but all of them showed that dyes cause the plants to develop in a more active way.

(3) *Green Nordstern Beans in Open Ground*:
(Fünphausen (G.) March-May 1937.)

Plot No.	Number of Plants	Treated with	Yield in Grams	Average per plant in grs.
Control	15	Plain water	1780	118.6
No. 1	15	1 gr. PhotoSensin diss. in 12½ l. water	2530	168.6
2	15	1 gr. PhotoSensin diss. in 15 l. water	2505	167
3	10	1 gr. PhotoSensin diss. in 17½ l. water	2460	246

Each m^2 (approx. 1 sq. yd.) received 5 liters of the PhotoSensin solution every 8-10 days. Between treatments the plot was amply watered. A comparison of the average yield of plant between the control plot and Plot No. 3 shows the proportion as 118.6:246. This is an *increase of* 107.4%.

This experiment shows that the bean experiment may be carried out in open ground with as good results as in pots.

B. *Experiment with Carrots*:
(L. M. McLaren Ranch, PhotoSensin Inc., Yuma, Arizona, 1939).

Treated in an open ground fertilized with ammonium gas. The water was applied by the irrigation method. PhotoSensin treatment began in January and was applied four times, each time one kg. of PhotoSensin powder was added to an acre. (This has to be tested, depending upon the plant and climate, respectively, under which it is applied. It varies usually between ½ and 2 kilos per acre.) The entire treated area consisted of 300 acres, out of which a small plot was afterward tested for exact weights of crop.

Total planted area (for this test):	14.84	gross acres
PhotoSensin treated	3.16	" "
Untreated	11.68	" "
From the untreated 11.68 acres—3826 crates or 327.6 crates per acre		
From the treated 3.16 acres—1393 " " 440.8	" " "	
Increase	113.2	" " "

Results: 34.6% more in weight of the PhotoSensin treated carrots.

C. *Experiment with Radishes, Asters and Chrysanthemums*:
(Dr. Sellei's Garden, Los Angeles, California, 1939.)

In two plots of 5 sq. yds. each, about one yard apart, *radishes* were sown from seeds, and two months old *chrysanthemums* and *asters* were

planted July 6, 1939. One of the plots (Plot 1) was treated with a PhotoSensin solution; the other (Plot 2) received the same amount of plain water (control). Plot 1 was first treated on July 7th and thereafter every ten days, until July 27th, thereafter every twelve-fourteen days as long as the plants bore flowers. The PhotoSensin solution was always prepared in the following manner: 1.5 PhotoSensin tablets (0.5 gr.)* were dissolved in three gallons of water and poured slowly on the 5 yard plot. After this was done, the 5 sq. yds. were *immediately* watered with 12-15 gallons of *plain* water. The quantity of water

Fig. 3. *Asters* treated with PhotoSensin and control (C), untreated.

always was as much as the soil was able to absorb. (After a rain the soil needs less water.)** On the days between two treatments both plots were watered as usual.

(a) The treated *radishes* were already much advanced the third week after sowing. The radishes were pulled up on August 19th. The treated radishes were much larger than the untreated ones. The weight of the whole plant was as follows:

10 Untreated radishes	70 grs.
10 Treated radishes	280 grs.

*In some other instances a smaller amount of PhotoSensin has proven better according to seed, soil and climatic conditions.

**How much water the soil will absorb should be determined by test. It is usually between 2-15 liters per sq. yd.

(b) The treated *asters* and *chrysanthemums* bloomed 8-14 days earlier, had more and larger blossoms; the plants were higher. (See photograph Asters). The blooms of the chrysanthemums were counted when they were in full bloom, showing: Controls, average:—20 blooms per plant; treated:—40 blooms per plant.

D. *Experiment with "Darwin" Tulips—large size—in pots*:
(Municipal Gardeners' Training School, Budapest, Hungary, 1937-1938.)

Tulip bulbs of roughly equal scale weight were chosen for this experiment. Twelve series of potted tulips, 10 pots each, were treated with various concentrations of PhotoSensin solution. A thirteenth and fourteenth series of 10 pots each, remained untreated and formed the controls. Each pot contained 300 grams of well fertilized soil and one tulip bulb. At each treatment the pots received 30% of the PhotoSensin solution calculated on the weight of soil in the pot, i. e.: 90 cm^3 per pot. They all were first treated in the fall, then placed in the dark on Dec. 6th where they remained until Jan. 24th, on which date they were removed to the hothouse. During this period they were treated three times:

(1) Oct. 15, 1937 (10 days after bulbs were placed in pots)
(2) Nov. 5, 1937
(3) Jan. 24, 1938

From that time on they were treated four additional times in 10-12 day intervals. The twelve treated series were the following:

a) 1 gram PhotoSensin powder dissolved in 10 l. plain water
b) 1 " " " " " 12½ " " "
c) 1 " " " " " 15 " " "
d) 1 " " " " " 17½ " " "
e) 1 " " " " " 20 " " "
f) 1 " " " " " 22½ " " "
g) 1 " " " " " 25 " " "
h) 1 " " " " " 27½ " " "
i) 1 " " " " " 30 " " "
j) 1 " " " " " 25 " " "
k) 1 " " " " " 20 " " "
l) 1 " " " " " 10 " " "

In the thirteenth and fourteenth series were the untreated control plants.

The result of this experiment is visualized in the accompanying illustration.

On the left are seen the untreated control plants, then in the rows the various treatments as mentioned from (a)-(l). In the last row on the right is another untreated series. It will be observed that, beginning

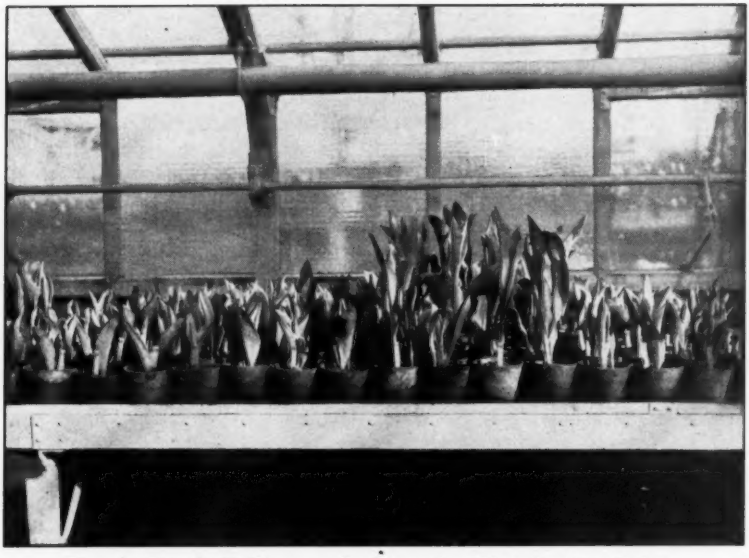

Fig. 4. *Darwin Tulips* treated with various PhotoSensin solutions.

Fig. 5. *Darwin Tulips* untreated, and treated with PhotoSensin.

from the concentration of (a) (1 gram PhotoSensin in 10 liters of water), the tulips were higher and higher, reaching a top in the concentrations (g) and (j), (h) and (i) (1 gram PhotoSensin in 25, 27½ and 30 lits. of water). In these the tulips were 30 cms. high. Thereafter the tulips are less high until they decline almost to the height of the control plants: 8-10 cms.

This experimental series shows in an interesting way how the different dye concentrations can influence the growth and development of tulips which are of equal size and quality and placed in the same quantity of soil.

The following data were observed:

	January 24, 1938	March 8, 1938
Height of Plants		
Control	8 cms.	30 cms.
Treated with 1:10*	15 "	
" " 1:25	30 "	67 "
Length of Bloom		
Control	—	3 "
Treated with 1:25	—	8 "
Width of Leaves		
Control	—	·5-6 "
Treated	—	8 "

Dwarfing Experiments:

In contrast to weak dilutions the high concentrations of fluorescent dyes (1-10 cm^3 of a ½-3% solution) lead to a dwarfing of the treated plants.

For simplifying this experiment, a powder "Dimin" which contains Fluorescein and bicarbonate of soda was mixed, to make this soluble, with excessively small amounts of copper and iron sulfate. If gradually increasing amounts of this powder are used, the result is smaller and smaller plants.

Dwarfing experiments with castor beans, for instance (Municipal Gardeners' Training School, Budapest, Hungary) showed the following results (see also illustration):

Sown: June 17, 1938.
Photo taken Sept. 7, 1938.
Results obtained in 81 days—all pots containing 500 grams of soil, treated twice:

*1:10 means 1 gram PhotoSensin powder dissolved in 10 liters of water
1:25 " 1 " " " " " 25 " " "

	Height of Plant
Control—untreated	48 cms.
Treated with "Dimin"	
1 powder (1 gram)	26 cms.
2 powders (2 grams)	22 "
3 powders (3 grams)	14 "

The leaves also became gradually smaller and smaller. All plants treated, and controls, had flowers, and later fruit.

Fig. 6. *Dwarfing of Castor Beans* treated with "Dimin" powder.
C = untreated.
D (1) = treated with three powders.
D (2) = treated with two powders.
D (3) = treated with one powder.

CONCLUSIONS

Many plants treated with a properly diluted fluorescent dye grew much faster than the untreated control plants: they bloomed earlier, their fruit ripened faster, and the yield increased from 20-100%, and even more. Sometimes fruit of extraordinary size was obtained. The rule seems to be, however, that increase is possible only in so far as it corresponds with the plant's nature (genotype). Each plant has a PhotoSensin-sensitive period. The PhotoSensin treatment must be applied just in this period, which must be ascertained for each plant by experiments; otherwise really good effects with dyes cannot be expected. The plants have their PhotoSensin-sensitive period, in most cases, at the same time that they have their light-sensitive period.

In dwarfing experiments carried out with strong dye solutions, ⅓-⅕ of the size of the control plants was obtained.

It is an interesting fact that plants in pots and flats treated with

fluorescent dyes can develop in a proper manner only if the soil is well fertilized. Reversely, the sensitized plant exhausts the foodstuffs of the soil in a relatively short time and afterwards, for lack of these, the plant cannot develop as usual and its growth remains behind the untreated control potted or flat plants. This also proves that plants treated with a highly diluted dye solution have an increased metabolism, as they require considerably more nutrients than the untreated plants.

This phenomenon is caused by the light-sensitizing effect of dyes on plants. The influence of the dyes on plant growth manifests itself by the photo-chemical action of these agents which produces an increase in their sensitiveness to light.

The effect of the fluorescent dyes can be increased with small amounts of copper and iron salts which always have to be considerably less than the dye itself.

The negative geotropism apparent after plants are treated with Eosin is, according to Boas, the result of an irritating action in the wake of which the growth-promoting substances in some part of the plant are stimulated, and retarded in some other part. Photo- and chemotropism manifest after using certain dyes are similar effects of an irritating action. Both may similarly cause the formation of growth-promoting substances which, for instance, are identical in both the phototropism and the length-growing process, and which are subject to important modifications during plant growth.

It has been found in many experiments that plants were promoted in their growth by the action of certain dyes. This may be explained as the result of the action taking place in the plant. This probably causes an increase in the growth-promoting substances (Boysen-Jensen, Kögl, W. F. Went, Van Overbeek, Thimann, etc.), (1) *auxin* (acid indolacetic, etc., and similar compounds), (root growths), (2) growth substances, *Vitamin* B_1 (thiamin chloride, etc.) and, it may be assumed—a more active physiological activity in the whole plant life. It is known that artificial light (Natrium, Neon-light) of strong intensity causes better assimilation in plants without influencing them to grow higher. The fluorescent dyes may be considered in the light of intermediary materials which can raise the light-sensitiveness of plants and increase their physiological activity and so their whole metabolism.

SUMMARY

1. The process of light-sensitizing is already well known. Light-sensitive materials can be rendered more sensitive with some proper dyes.

2. As the plant has an especially light-sensitive substance, and the whole plant may be considered as light-sensitive (see Phototropism).

this light-sensitiveness of the plant can be increased by certain dyes, in the first place, with fluorescent dyes.

3. With a weak concentration of these certain fluorescent dyes the plant becomes irritated, which stimulates the growth-promoting substances and calls forth a faster development of the plant.

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APPLICATION OF STATISTICS TO PROBLEMS IN BACTERIOLOGY

V. THE PROBABILITY OF OCCURRENCE OF VARIOUS EXPERIMENTAL RESULTS

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(*Received for publication May 2, 1940*)

When a bacteriologist wishes to make a determination of the bacterial population of a suspension, he may do so with one of three methods. He can make a plate count, a direct count, or a dilution count. In any case, he usually has to dilute the suspension to such an extent that the density is low enough for counting. In the plate count, a 1 cc. sample from each of several dilutions is inoculated into separate tubes of melted nutrient agar. The inoculated tubes are then poured into plates. These are incubated until colonies appear, and from the number of colonies, the original population can be computed. In the direct count, a definite volume of a dilution is spread over a measured area on a glass slide, and individual cells are counted by direct microscopic examination. These two methods present no unusual problems, and the figures obtained can be handled by any of the ordinary statistical methods. The dilution method, however, presents some problems peculiar to itself, not only in respect to laboratory technique, but also in the interpretation of resulting data. It is this method which we are concerned with in this paper.

In many bacteriological problems, any one of the three methods may be as applicable as either of the other two. There are some problems, however, in which the advantages of one procedure over another are considerable. For example, the dilution method has been found to be more reliable in determining the number of organisms of a particular kind in a mixed culture, or in dealing with organisms that do not grow readily on solid media. In some cases, the dilution method is the only available means of determining population. It is important, therefore, that a bacteriologist have available the tools necessary for a proper analysis of data obtained by this method of evaluating bacterial population.

The procedure in the dilution method is usually as follows: From the original suspension one makes a series of dilutions in powers of 10, using sterile water or sterile salt solution. One cc. samples from each dilution are inoculated into tubes containing a suitable liquid medium. The tubes are then incubated, and the results are recorded in terms of

the number of tubes showing growth in each dilution. From previous work, it has been fairly well demonstrated that not much reliance can be placed on data from fewer than 10 tubes for each dilution. Usually only three dilutions are critical. In the lowest, all or nearly all the tubes will show growth, and in the highest, most of the tubes will have no growth. In the literature on this subject, the result is commonly referred to as the code (McCrady, 1915; 1918). For example, a code of 10-5-0 from dilutions of 10^5, 10^6 and 10^7 would mean that all the tubes inoculated from the 10^5 dilution, 5 of the ten tubes inoculated from the 10^6 dilution, and none of the ten tubes from the 10^7 dilution show growth. By means of probability equations, it is then possible to calculate the "most likely" population corresponding to the code obtained. Equations suitable for such calculations can be derived from Poisson's theorem (Greenwood and Yule, 1917; Halvorson and Ziegler, 1933). For the case where 10 tubes are used in each of the three critical dilutions, it can be shown that the following equation applies:

$$P = \frac{10!}{p_1!q_1!}(1-e^{-10ax})^{p_1}(e^{-10ax})^{q_1} \cdot \frac{10!}{p_2!q_2!}(1-e^{-ax})^{p_2}(e^{-ax})^{q_2} \cdot \frac{10!}{p_3!q_3!}(1-e^{-.1ax})^{p_3}(e^{-.1ax})^{q_3} \qquad (1).$$

p_1 = number of tubes showing growth inoculated with 10 cc.
p_2 = number of tubes showing growth inoculated with 1 cc.
p_3 = number of tubes showing growth inoculated with 0.1 cc.
q_1 = number of tubes showing no growth inoculated with 10 cc.
q_2 = number of tubes showing no growth inoculated with 1 cc.
q_3 = number of tubes showing no growth inoculated with 0.1 cc.
x = number of bacteria per cc.
a = dilution factor. It may have values of 10^0, 10^{-1}, 10^{-2}, 10^{-3} etc.

From this equation it is evident that the probability of obtaining a given code will depend upon the density of the population under investigation. The following graph is a plot of this equation for code 10-5-0.

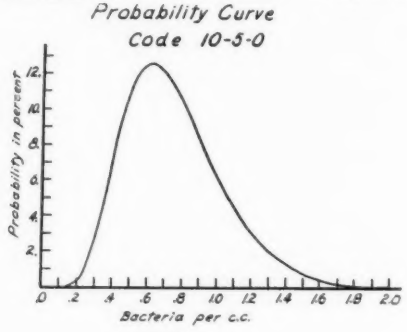

Fig. 1

Most investigators in this field have selected the "most likely" population as the best estimate (Greenwood and Yule, 1917; Halvorson and Ziegler, 1933; McCrady, 1915; 1918). Some years ago, there was published from this department a series of tables giving the most likely number of organisms per cc. for each of the likely codes when 10 tubes are used in each of 3 dilutions. Tables were also presented to simplify the evaluation of the most likely population for other codes not included, particularly those obtained when more or fewer than 10 tubes are used in each dilution.

Various investigators have also examined the reliability of the most likely population as determined from the various codes (Greenwood and Yule, 1917; Halvorson and Ziegler, 1933a; 1933b; McCrady, 1915; Reed, 1925; Stein, 1919; Wohlman and Weaver, 1917). Since the frequency distribution in this case is skewed, the standard deviation is not particularly useful. Therefore, instead of the standard deviation, these investigators have determined an upper and a lower limit within which the actual population of the unknown suspension can be reasonably assumed to fall. These limits may be evaluated by two different methods. From the equation expressing the frequency distribution for any one code, one can, following Bayes' principle (Gordon, 1939 and McCrady, 1915) determine upper and lower limits that will include, for example, 97% of the total frequencies. This has been termed by some the method of inverse probability, and its reliability has been questioned. One may also arrive at these limits by following the usual procedure in statistical analysis. One may imagine, for example, that a very large number of analyses are made on a single suspension, and determine from the general equations the frequency distribution of all possible most likely populations. Analyzing such data statistically, one can determine the standard deviation and also the upper and lower limits that will include, for example, 97% of the total frequencies (Halvorson and Ziegler, 1933b; Matuszewski, Neyman, and Supinska, 1935; Pearson, 1939). In contrast to the above-mentioned method, this one is called the method of direct probability. In the former case we are concerned with the frequency with which a given code can be obtained from a universe of suspensions in which all bacterial populations are equally probable. In the second case, we are making inquiry into the frequency with which the various codes (or the various most likely populations) can be obtained from a single suspension. A comparison of the two methods has not yet been made available in the literature, so it cannot be stated just how closely these two methods agree, but various statisticians have expressed the opinion that the two methods will give results that are in substantial agreement.

Another problem suggests itself in this connection, and that is the probability of occurrence of the different codes. This is the problem

with which we are chiefly concerned in this paper. In our previous publications (1933; 1935) we have given, in addition to the most likely populations, the probability of occurrence of each code, assuming that the population in the suspension from which the samples were taken was the most likely population. These probabilities were included primarily to serve as indications of the likelihood of occurrence of the various codes. In publishing our tables, we included only those codes having a probability of occurrence greater than .0001.

A suspension containing the most likely number of organisms obviously provides the most favorable conditions for a certain code to appear. In any actual analysis, one will very seldom have a suspension with a population equal to the most likely population for a given code. Therefore, the probability of getting a certain code will in most instances be lower than that indicated in our previous tables. It seemed to us, therefore, that we should calculate the probability of occurrence of the various codes irrespective of population. Let us suppose, for example, that a large number of investigators collect data from dilution counts. On examination of such data, we should find that certain codes occur more frequently than others, and if we should evaluate the frequency for each one, and its corresponding probability, we would have the probability which we are now attempting to calculate. The sum of all the probabilities of all the codes would in this instance be equal to 1, or 100%.

In approaching this problem, one must realize that any given code, such as 10-5-0, can occur at different positions on the dilution scale. However, in solving the problem, we are interested only in the probability of its occurrence and not in its position. Table I shows how this code may be obtained with different suspensions having populations differing by approximately powers of 10.

TABLE I

CODE OF THE SAME RELATIVE POPULATION AT DIFFERENT DILUTIONS

Dilution:	10^{-0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Suspension I	10	5	0	0	0	0
II	10	10	5	0	0	0
III	10	10	10	5	0	0
IV	10	10	10	10	5	0

If equation 1 is applied to each of these suspensions, a separate curve will be generated for each one, even though the code is the same. Therefore, for any given code, there will be generated a family of curves, as illustrated in Fig. 2.

In this family of curves, there is a definite degree of similarity. The maxima have all the same value of P; the most likely value of x for any one of the curves is ten times greater than that of the preceding curve;

the area under any one curve is ten times the area under the preceding curve; the value of P at the point of intersection of two adjacent curves will be the same for all other corresponding intersections. If we select any point on one curve, the value of x at that point is ten times the value of x for the corresponding point on the preceding curve.

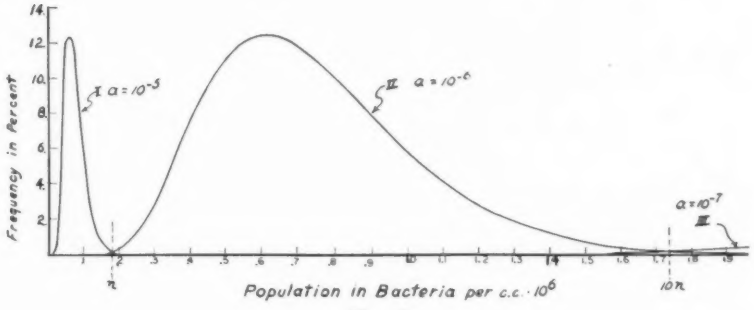

Fig. 2

It is apparent that the probability we are interested in is the average value of the ordinates for this family of curves. It can be seen by an inspection that this average value of the ordinate will depend not only upon the characteristics of any one curve but also upon the point of intersection of adjacent curves. For example, if we have a suspension with a population n (the value of x at the point of intersection of two adjacent curves), the probability of occurrence of this code is not the value of P at this point, but rather twice the value of P, because with this suspension, the code can be obtained two different ways, each having an equal probability.

Theoretically, this problem can be solved by the use of Calculus. If we should integrate all the curves from zero to infinity, so as to get the area under each curve, we could add all these areas and obtain the total area. By dividing this sum by the length of the abscissa, we would obtain the average ordinate. In considering the problem from this point of view, however, it is apparent that we will be dealing with indeterminates both in the numerator and in the denominator because the area under the last curve will be infinite, and its abscissa will have an infinite length. It is apparent, therefore, that we cannot deal with the entire family of curves, but must confine ourselves to a finite portion of the x-axis. The problem is what portion of the x-axis to use. It can still be solved by Calculus if we take two limits on the x-axis, from K to 10K, and find the area of each of the curves occurring between these two limits. By adding all of these areas, and dividing by 9K or

(10K-K), we should get the average ordinate over this portion of the x-axis. Before we can definitely state that this solution will give us the correct answer, we must know whether or not the value obtained will depend upon the value of K used.

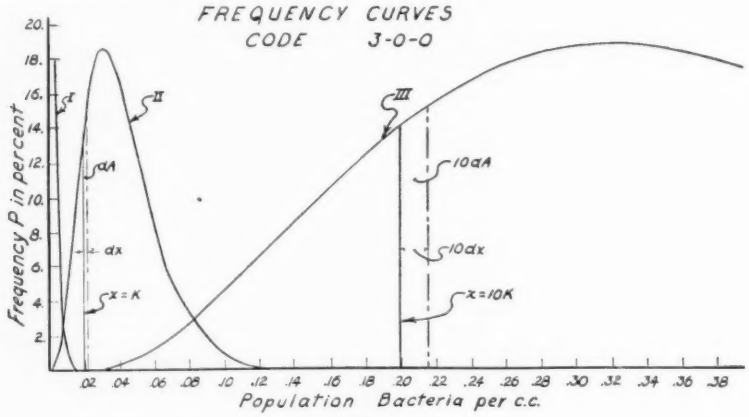

Fig. 3

Referring to Fig. 3.

Let A equal total area under all curves between $x = K$ and $x = 10K$.

$$\text{Average } P_{a1} = \frac{A}{10K - K} = \frac{A}{9K}$$

Let us increase the lower limit by dx. The new limit then becomes $x = (K + dx)$ and $x = 10(K + dx)$.

The new area becomes

$$A - dA + 10dA = A + 9dA.$$

$$\text{Average } P_{a2} = \frac{A + 9dA}{10(K + dx) - (K + dx)} = \frac{A + 9dA}{9K + 9dx}$$

Let $P_{a2} = z\ P_{a1}$, where z is equal to unity if $P_{a2} = P_{a1}$
is a number smaller than unity if $P_{a1} > P_{a2}$
is a number larger than unity if $P_{a1} < P_{a2}$

$$\text{Then} \quad \frac{A + 9dA}{9K + 9dx} = z\frac{A}{9K}$$

From this it can be shown that

$$z = 1, \text{ if } \frac{dA}{dx} = \frac{A}{9K} \text{ or when } P = P_{a1}.$$

It is evident from this that if P_a is determined for different limits, its value will not be the same. P_a will have a maximum or minimum value when $P_a = P$.

The following Table II shows how the value of P_a changes for code

TABLE II
VARIATIONS IN THE AVERAGE VALUE OF P
FOR DIFFERENT LIMITS OF CODE 3-0-0

Limits	P_a
.01 to .1	.1094
.015 to .15	.0975
.02 to .2	.1031
.03 to .3	.1248
.04 to .4	.1385
.05 to .5	.1432
.06 to .6	.1389
.07 to .7	.1314
.08 to .8	.1225
.09 to .9	.1154
.10 to 1.0	.1094

3-0-0 for different values of the limit K. Figure 4 is a curve showing a plot of these data.

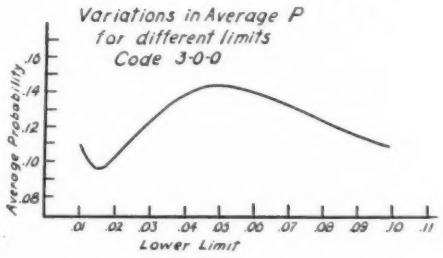

Fig. 4

It can be seen from the above table and graph that, in the case of code 3-0-0, P_a has a minimum value of .0975, and a maximum value of .1432, with an average of about .122. It seemed to us logical to assume that this would be the probability desired. This value would have been obtained if all the curves were integrated between the limits .00775 and .0775, and the total area were divided by (.0775 — .00775) or 9 .0775.

Figure 5 shows the various curves for code 3-0-0. In this figure is also plotted a composite of the individual curves in the family. The heavy broken line shows how P_a varies with different limits. The average value of P_a, 12.2%, is indicated on the graph by a straight line. Although this is the probability we desire to obtain, it is evident that it would be extremely difficult to calculate this probability for all the codes in the same manner as we have for code 3-0-0. It would seem, from Fig. 5, that the value of K that should be used to give the probability desired would be one equal to a value of x slightly smaller than that corresponding to the maximum value of P in the composite curve, or

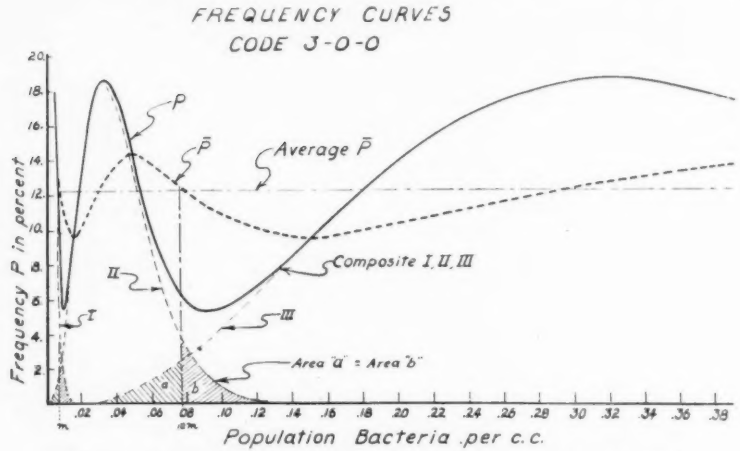

Fig. 5. (P with a line over it is the equivalent of P_a in the text.)

one slightly smaller than the one corresponding to the minimum value of P in the composite curve. It would be difficult to determine this value for each of the codes, and once determined, it would also be very difficult to integrate the family of curves between these limits. We therefore felt that it was necessary to find a more direct method of determining the average value of P_a. We did this by graphical means. In Fig. 5, we selected a value of $x = 10$ m such that an area (a) was cut off under curve III from $x = 0$ to $x = 10$ m equal to the area (b) under curve II from $x = 10$ m to $x = \infty$. It can be seen that the sum of the areas under all the curves of the family from the limits m to 10 m will be the same as the area under curve II from the limits $x = 0$ to $x = \infty$. This latter area can be found with less difficulty than the areas under all the curves from m to 10 m. If the area under curve II

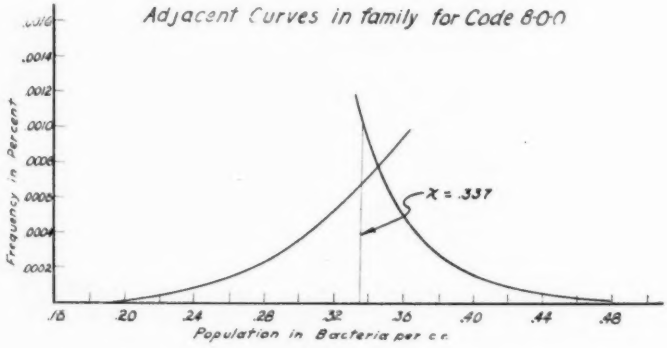

Fig. 6

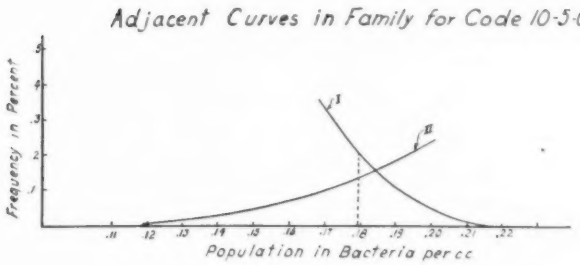

Fig. 7

is now divided by the length of the ordinate, namely (10m — m), one will obtain a value for the average ordinate P_a that is only slightly greater than that desired. The value of m is found by plotting the legs of two adjacent curves, and determining the equal areas by means of a planimeter. Figures 6 and 7 show the areas, and the values of m for the two codes 8-0-0 and 10-5-0. Proceeding in this manner, we determined the probability of occurrence of the codes that are given in Table III.

TABLE III

THE PROBABILITY OF OCCURRENCE OF BASIC CODES

	Area by Integration			P_a = (area ÷ 9m.)	
Code	from x = o to x = ∞	m	9m	Calculated	Corrected
1-0-0	.00892	.00240	.02160	.4130	.4025
2-0-0	.00882	.00485	.04365	.2021	.1969
3-0-0	.00871	.00775	.06975	.1249	.1218
4-0-0	.00859	.01105	.09945	.0864	.0843
5-0-0	.00845	.01500	.13500	.0626	.0610
6-0-0	.00828	.01950	.17550	.0472	.0460
7-0-0	.00808	.02560	.23040	.0351	.0342
8-0 0	.00782	.03370	.30330	.0258	.0252
9-0-0	.00745	.04600	.41400	.0180	.0176
10-0-0	.00677	.06950	.62550	.0108	.0105
			Total:	1.0259	1.0000

If we examine these codes, it can be seen that code 1-0-0 includes a large number of codes. It is really the series P_1-P_2-P_3 . . . . . 1-0-0. In fact, it will include all codes that end in 1. Likewise, code 2-0-0 will include all codes that end in 2, etc. It is apparent from this that the short table above takes in all possible codes, and therefore, if the correct limits had been used, the sum of their probabilities would add up to 1.0000.

Not much error will be made by assuming that our method of selecting the integrating limits will produce the same percentage error in each of the calculated values for P_a. We therefore corrected each of the above by reducing them to the extent of 2.52%.

TABLE IV

FREQUENCY OF OCCURRENCE OF DIFFERENT CODES

Code	Probability	Totals I	Totals II
10 10 10 10 0 0	.0105	.0105	.0105
10 10 10 9 0 0	.0175	.0175	.0176
10 10 10 8 0 0	.0250	.0250	.0252
10 10 10 7 0 0	.0340	.0340	.0342
10 10 10 6 0 0	.0434		
10 10 9 6 0 0	.0021		
10 10 8 6 0 0	.0005	.0460	.0460
10 10 10 5 0 0	.0525		
10 10 9 5 0 0	.0054		
10 10 8 5 0 0	.0015		
10 10 7 5 0 0	.0005	.0599	.0610
10 10 10 4 0 0	.0612		
10 10 9 4 0 0	.0134		
10 10 8 4 0 0	.0053		
10 10 7 4 0 0	.0026		
10 10 6 4 0 0	.0011		
10 10 5 4 0 0	.0005	.0841	.0843
10 10 10 3 0 0	.0618		
10 10 9 3 0 0	.0250		
10 10 8 3 0 0	.0143		
10 10 7 3 0 0	.0085		
10 10 6 3 0 0	.0056		
10 10 5 3 0 0	.0034		
10 10 4 3 0 0	.0015		
10 10 3 3 0 0	.0008	.1209	.1218
10 10 10 2 0 0	.0515		
10 10 9 2 0 0	.0359		
10 10 8 2 0 0	.0279		
10 10 7 2 0 0	.0222		
10 10 6 2 0 0	.0177		
10 10 5 2 0 0	.0122		
10 9 5 2 0 0	.0005		
10 10 4 2 0 0	.0093		
10 9 4 2 0 0	.0007		
10 8 4 2 0 0	.0005		
10 10 3 2 0 0	.0062		
10 9 3 2 0 0	.0010		
10 8 3 2 0 0	.0005		
10 10 2 2 0 0	.0035		
10 9 2 2 0 0	.0009		
10 8 2 2 0 0	.0005		
10 10 1 2 0 0	.0019		
10 9 1 2 0 0	.0009		
10 8 1 2 0 0	.0005	.1943	.1969
10 10 10 1 0 0	.0301		
10 10 9 1 0 0	.0340		
10 10 8 1 0 0	.0365		
10 10 7 1 0 0	.0373		
10 10 6 1 0 0	.0374		
10 9 6 1 0 0	.0008		
10 10 5 1 0 0	.0357		
10 9 5 1 0 0	.0048		
10 10 4 1 0 0	.0319		
10 9 4 1 0 0	.0056		
10 8 4 1 0 0	.0009		
10 10 3 1 0 0	.0257		
10 9 3 1 0 0	.0067		
10 8 3 1 0 0	.0020		
10 7 3 1 0 0	.0010		
10 6 3 1 0 0	.0006		
10 10 2 1 0 0	.0203		
10 9 2 1 0 0	.0092		
10 8 2 1 0 0	.0041		
10 7 2 1 0 0	.0031		
10 6 2 1 0 0	.0023		
10 5 2 1 0 0	.0008		
10 4 2 1 0 0	.0005		
10 10 1 1 0 0	.0103		
10 9 1 1 0 0	.0075		
10 8 1 1 0 0	.0059		
10 7 1 1 0 0	.0046		
10 6 1 1 0 0	.0034		
10 5 1 1 0 0	.0030		
10 4 1 1 0 0	.0020		
10 3 1 1 0 0	.0009		
10 10 1 0 1 0	.0005		
10 9 2 0 1 0	.0005		
10 10 2 0 1 0	.0007		
10 10 3 0 1 0	.0016		
10 9 3 0 1 0	.0014		
10 10 4 0 1 0	.0017		
10 9 4 0 1 0	.0013		
10 10 5 0 1 0	.0025		
10 9 5 0 1 0	.0008		
10 10 10 0 1 0	.0027		
10 10 9 0 1 0	.0031		
10 10 8 0 1 0	.0034		
10 10 7 0 1 0	.0034		
10 10 6 0 1 0	.0032	.3957	.4025
Totals:		.9879	1.0000

We then proceeded with the other codes, such as 10-4-0, 9-4-0, 8-4-0, etc., calculating the value of the probability as indicated above, and correcting the results as for the codes above. This was done for all the codes having a probability equal to or greater than 0.0005. The results are given in Table IV.

In this table are given two columns of totals, I and II. In column I are the totals of the probabilities of the individual codes that have been calculated, while in column II are the corrected values from Table III. For example, the individual codes that end in 1 have probabilities that add up to 0.3957. The difference between this and 0.4025 represents the probability of occurrence of all the other codes ending in 1 that are not included in this table. Each of these will occur less frequently than 0.0005. The total of the probabilities for all these codes is 0.9879. In

TABLE V

COMMONLY OCCURRING CODES

Code						Probability	
10	10	10	10	0	0	.0105	
10	10	10	9	0	0	.0175	
10	10	10	8	0	0	.0250	
10	10	10	7	0	0	.0340	
10	10	10	6	0	0	.0434	
10	10	10	5	0	0	.0525	
10	10	10	4	0	0	.0612	
10	10	9	4	0	0	.0134	
10	10	10	3	0	0	.0618	
10	10	9	3	0	0	.0250	
10	10	8	3	0	0	.0143	
10	10	7	3	0	0	.0085	
10	10	10	2	0	0	.0515	
10	10	9	2	0	0	.0359	
10	10	8	2	0	0	.0279	
10	10	7	2	0	0	.0222	
10	10	6	2	0	0	.0177	
10	10	5	2	0	0	.0122	
10	10	4	2	0	0	.0093	
10	10	10	1	0	0	.0301	
10	10	9	1	0	0	.0340	
10	10	8	1	0	0	.0365	
10	10	7	1	0	0	.0373	
10	10	6	1	0	0	.0374	
10	10	5	1	0	0	.0357	
10	10	4	1	0	0	.0319	
10	10	3	1	0	0	.0257	
10	10	2	1	0	0	.0203	
10	9	2	1	0	0	.0092	
10	10	1	1	0	0	.0103	
10	9	1	1	0	0	.0075	85.97

other words, the probability of occurrence of all the other possible codes is 0.0121.

When 10 tubes are used in each dilution, there are therefore about 100 significant codes. In Table V, are listed the codes that will occur with a frequency of about .01 or greater. Of these, there are only 31 separate codes, having a total probability of about 0.86. These are the codes that will usually be obtained in experimental results.

While it is not impossible to get codes not included in Table IV, one may if he gets such codes, justly suspect that contamination may be responsible. It has also been our experience that if a medium is used which is unfavorable for growth, these infrequent codes appear with considerable regularity. This phase of the problem requires further investigation to determine whether an unfavorable medium might bring about such results.

It should be borne in mind that the probabilities which have been calculated are based upon the assumption that all bacterial populations are equally probable. If a person should wish to check these probabilities by experimental results, he should bear in mind that his data will have to be obtained from suspensions selected at random, rather than from a single suspension where the population is necessarily fixed. In this problem, chance plays a dual role---first, in the selection of the suspension from a universe of suspensions, and second, in the determination of the code from the suspension selected.

SUMMARY

We have calculated the probability of occurrence of various combinations of tubes showing growth and no growth where ten tubes are used in each of three critical dilutions in the determination of bacterial populations by the dilution method.

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MITOTIC INDICES IN REGENERATING URODELE LIMBS

II. A STUDY OF THE DIURNAL DISTRIBUTION OF CELL DIVISIONS[1]

By

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(*Received for publication May 6, 1940*)

In a previous paper on urodele limb regeneration, (Litwiller, 1939), data were presented indicating a relatively high mitotic rate at the base of the regenerate during early stages of limb regeneration and a peripheral shift of the region of high mitosis to more distal levels as regeneration progressed. In that work, no particular effort was made to check the time of day when the various regenerated limbs were removed and fixed. It seemed desirable to check this matter, particularly in view of work by Carleton (1934) on white mice which had indicated a definite daily rhythm in cell division. This was done, keeping the animals under controlled conditions of light and darkness and removing the regenerated limbs at various definite time intervals.

MATERIALS AND METHODS

The newt, *Triturus pyrrhogaster* (Boie), was again used. The right anterior limbs of 90 animals were amputated, care being taken that the humerus did not protrude from the wound. The stumps were allowed to regenerate for 55 days. Animals were divided into three groups: 1) those kept in the ordinary day and night rhythm of light and darkness; 2) those kept in constant darkness in a dark chamber; 3) those kept in constant light (60 watt electric light bulb suspended 5 feet above the container). The experiment was run from March 6th to April 30th in steam heated rooms, but not under constant temperature conditions. The regenerated limbs were removed and fixed in Bouin's solution at four different hours of the day: a) 6 A. M., b) 12 A. M., c) 6 P. M., and d) 12 P. M. The regenerates were sectioned at right angles to the main limb axis, eight μ in thickness and stained with Heidenhain's Haematoxylin. Separate counts were made of the total numbers of

[1]This work was aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

[2]The author wishes to express his appreciation to Dr. Paul Weiss of The University of Chicago for valued criticism and the use of the laboratory during the summer of 1939.

mesenchyme cells and of epithelial cells in any given cross section. In addition, the number of metaphase stages in the same sections were recorded. From these data, the ratio of mitotic to resting cells ("mitotic index") could be computed. Every 12th and 13th serial section was counted, thus supplying eight sets of data per mm. of the limb axis, from the base to the distal end of each limb. The data presented in this paper represent complete counts of eight regenerates in each of the three experimental series, i. e., a total of twenty-four animals.

RESULTS

The graph, Figure 1, presents the data on both mesenchyme and epithelium.

The abscissa is divided into four parts, corresponding to the four different times of limb removal during the twenty-four hour period. The ordinate represents the mitotic index expressed in percent. The solid lines represent mesenchyme while the broken lines indicate mitotic rates for epithelium. The various points plotted in any one series, e. g. day and night, represent the mean of all levels counted, not only of one regenerate, but of all the regenerates removed at that particular time. The ordinate represents, from left to right, the 24 hour time sequence of removal.

When the mitotic indices of all the levels counted within any given limb were compared among limbs removed at different times of day, no consistent trend was indicated. The fluctuation between the various limbs removed at a given time, e. g. 6 A. M., was as great as changes between the different groups (6 A. M., 12 A. M., 6 P. M. and 12 P. M.). However, if all the mitotic index counts from a given limb are averaged and combined with averages from other limbs similarly treated, the final mean obtained from both mesenchyme and epithelium of limbs removed at a definite time, present data of some interest. Each point in the graph, Figure 1, represents the average of from 60 to 90 separate mitotic indices. Sample cell counts from which each separate index was obtained are as follows: 17/836; 10/720; 5/554 etc. along the limb axis, the numerator representing the number of cells in metaphase, and the denominator the total cell count.

It will be noted that in all three series, the epithelial and mesenchyme indices, in general, fluctuate in a parallel fashion. In some cases the degree of fluctuation varies considerably.

The day and night series, as well as the constant darkness series, Figure 1, both indicate a peak of mitotic activity near 12 A. M., with a low point at night. The constant light series, in contrast, suggest

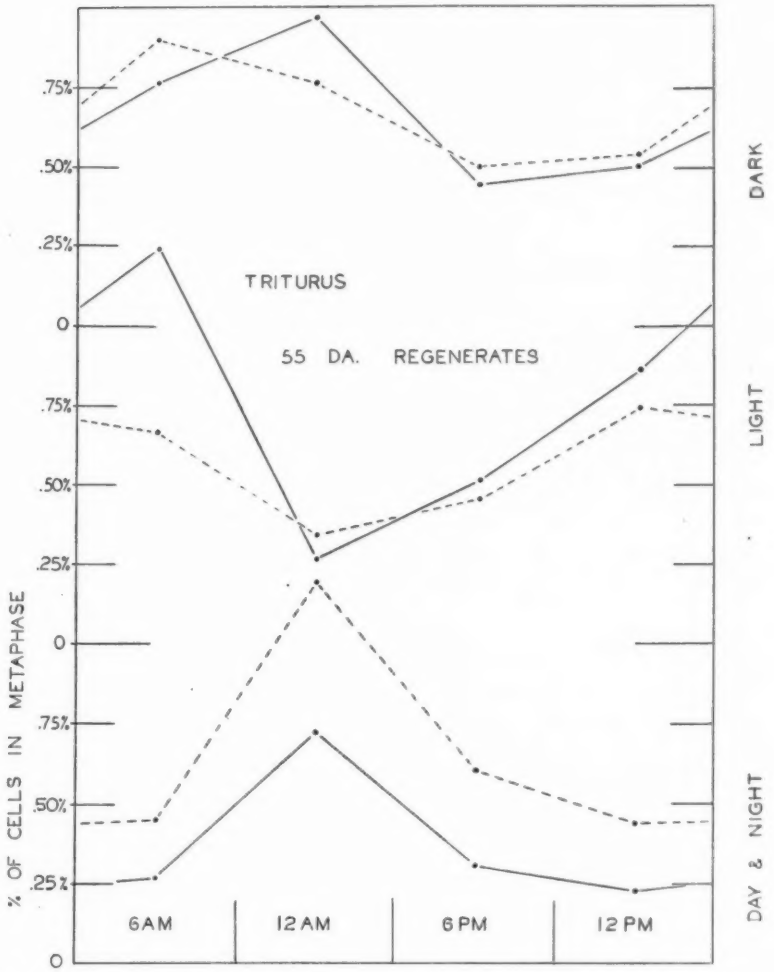

Fig. 1. Graph of diurnal distribution of mitotic indices in regenerating limbs.

more mitotic activity during the night. It is possible that temperature differences might be responsible for the changes appearing in the one series as against the trends indicated in the others. This possibility cannot be ignored since the experiments were not run under controlled temperature conditions. However, all three series were carried through in the same laboratory in adjoining rooms so that temperature differences between the various series would be of random character.

SUMMARY

The data obtained indicate a possible daily rhythm in the rate of mitotic activity in regenerating urodele limbs (55 days after amputation). Animals subjected to the normal alternation of light and darkness as well as those kept in constant darkness suggest a high rate of division at noon and a low rate at night. The constant light series also indicate a daily rhythm, but the peak occurs at night rather than at noon.

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ZUR FRAGE NACH DER ZUVERLÄSSIGKEIT GEWISSER BEWEISE FÜR DIE EXISTENZ DER MITOGENETISCHEN STRAHLUNG

von

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(*Received for publication June 6, 1940*)

ERSTER ABSCHNITT

Die Existenz der mitogenetischen Strahlung ist seit Jahren eine viel umstrittene Frage. Einerseits behaupten Gurwitsch und seine Schüler, sowie einige andere Forscher, unverkennbare Belege für die Wirkung dieser Strahlung beibringen zu können; sie beschreiben sogar deren Einzelheiten wie Wellenlänge und Spektrum. Andere Autoren dagegen konnten diese Resultate nicht bestätigen und meinen, deswegen die Existenz der mitogenetischen Strahlung anzweifeln zu müssen.

In Holland war es vor allem weiland Prof. Dr. L. K. Wolff, der sich diesem Arbeitsfelde widmete. Zusammen mit Frl. G. Ras hat er die sogenannte „slide-cell"-Methode ausgearbeitet, bei welcher man die Strahlung mit der Wirkung, die sie auf Bakterien haben sollte, nachzuweisen versucht.

In einer Veröffentlichung über eine von mir angestellte Nachprüfung der Zuverlässigkeit dieser Methode, habe ich ausführlich auf gewisse Umstände hingewiesen, welche die Schlussfolgerungen der mit ihr erzielten Resultate beeinflussen können. Beim Lesen neuzeitlicher Literaturbesprechungen über mitogenetische Strahlungsforschung fällt es mir auf, dass meinen Einwänden gar keine Rechnung getragen wird; meine Arbeit scheint ungenügend bekannt, und überdies oftmals nicht ganz verstanden worden zu sein, sodass es mir angebracht erscheint, Einiges noch etwas deutlicher zu sagen. Auch bedürfen meine damaligen Betrachtungen einer gewissen Erweiterung. So möchte ich hier diese Angelegenheit nochmals erörtern, obwohl ich nicht mehr in der Lage bin, mich diesem Thema experimentell zu widmen.

Meine Arbeit handelte vor allem von einer Nachprüfung der „slide-cell"-Methode von Wolff und Ras. Bei dieser Methode kommt es bekanntlich darauf an, die Vermehrung einer Bakterienzahl festzustellen, welche als Folge einer Bestrahlung auftreten sollte. Zu diesem Zwecke wird *Staphylococcus pyogenes aureus* einer jungen, während 15 Stunden bebrüteten Agarkultur entnommen, in flüssigem, serum haltigem Medium suspendiert, und als Detektor angewandt.

Zur Feststellung der Tauglichkeit der zu benutzenden Ingredienzien

wird gerinnende Milch als Strahler empfohlen. Diese kommt nebst einem gewissen Zusatz von Labpulver in ein Reagenzgläschen von Quarz, wonach die Detektorsuspension dem Einfluss dieses Strahlers ausgesetzt wird. Diese Versuchsanordnung wird in Abbildung 1 wiedergegeben.

Vor und während der Bestrahlung werden mittels steriler Pasteur-Pipette in verschiedenen Augenblicken der Detektorsuspension Proben entnommen und auf einer mit Paraffin bestrichenen Glasplatte steril aufbewahrt. Nach einer guten Viertelstunde werden aus jeder dieser Portionen mittels einer kalibrierten Mikropipette (Abb. 2a) eine Serie endgültiger Proben zur Bestimmung der darin befindlichen Kokken abgemessen. Dabei ist es natürlich wichtig, nach Möglichkeit für gute Mischung und kleine Messfehler zu sorgen.

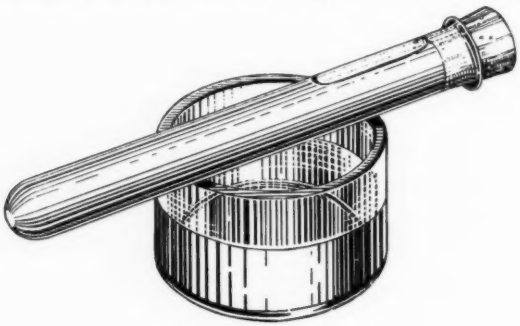

Abb. 1

Die Keimzahlbestimmungen werden nach einer von Wright (1923) eingeführten Mikromethode vorgenommen, wobei man sich sogenannter „slide-cells" bedient. Diese „slide-cells" werden aus zwei Objektgläsern angefertigt, zwischen die in regelmässigen Abständen fünf mit Vaseline imprägnierte Papierstreifen derartig gelegt werden, dass die Objektgläser einander nicht berühren, wodurch vier äusserst enge, sterile Kammern entstehen (Abb. 3).

In diese Kammern werden nun die endgültigen Suspensionsproben übergeführt und, nachdem die Ränder mit Paraffin verschlossen sind, auf 15 Stunden bei 37°C in den Brutschrank gestellt. Die Staphylokokken zeigen die Eigentümlichkeit, sich in den engen Kammern der „slide-cells" zu ziemlich gut begrenzten, und daher zählbaren Kolonien zu entwickeln, obwohl sie sich in einem flüssigen Medium befinden.

Beim Auszählen der „slide-cells" wird die mittlere Keimzahl der unbestrahlten Probenserie mit derjenigen der bestrahlten Serie verglichen, wobei eine statistisch gesicherte Vermehrung als Symptom für die Existenz der Strahlen aufgefasst wird.

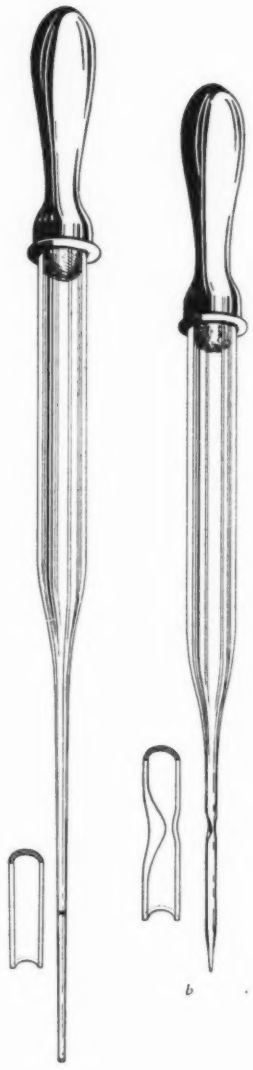

Abb. 2

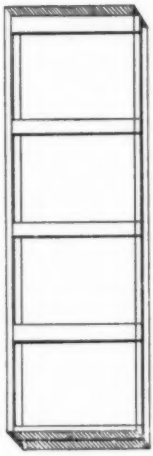

Abb. 3

Viele Misserfolge und Enttäuschungen habe ich mit dieser Arbeitsweise erfahren, trotz peinlich genauer Einhaltung der Methode, wie sie im Laboratorium von Prof. Wolff in Utrecht üblich war, als ich mich in die Praxis des Experimentes einübte. Um etwaige Differenzen in den Versuchsbedingungen zu beseitigen, gebrauchte ich zuletzt sogar die Ingredienzien des Utrechter Laboratoriums, was mir durch das grosse Wohlwollen von weiland Prof. Wolff ermöglicht wurde. Auch die Verhältnisse auf meinem Arbeitstisch waren den Utrechtern durchaus ähnlich. Allerdings hatte ich noch einige Vorsichtsmassregeln gegen das Eintrocknen der Suspensionstropfen auf den mit Paraffin bestrichenen Glasplatten und gegen zu grosse Messfehler bei der Probenentnahme getroffen. Auch wurde der P_H der Detektorsuspension nötigenfalls mittels K_3PO_4 justiert, schliesslich die Durchlässigkeit des von mir verwendeten Quarzgutes für ultraviolettes Licht im physikalischen Laboratorium der Amsterdamer Universität spektrographisch geprüft. Zuletzt waren übrigens weder Prof. Wolff noch Frl. Ras imstande, auch nur einen Unterschied zwischen der Utrechter und meiner Amsterdamer Durchführung der Experimente aufzuzeigen. Trotz alledem habe ich keine befriedigenden Ergebnisse erzielen können.

Die Schwierigkeiten waren zweierlei Art; es ergab sich einerseits kein reproduzierbarer und statistisch nachweisbarer Effekt, andererseits war die Streuung meiner Zahlen, der Ansicht von Prof. Wolff nach, viel zu gross.

Hinsichtlich des Ausbleibens des reproduzierbaren Effektes sei bemerkt, dass sich wohl eine Vergrösserung der Keimzahl einige Male spüren liess. Als ich aber dann auf genau dieselbe Weise den Versuch wiederholte, um das Phänomen mit umfangreicherem Zahlenmaterial einwandfrei festzustellen, versagte der Effekt völlig[1]. Darum bin ich der Meinung, dass man solche Daten nicht als Beweise für die Existenz der mitogenetischen Strahlen verwerten dürfe.

Im Anschluss an das Obige sei erwähnt, dass seit Mai 1935 auch im Laboratorium von Prof. Wolff der „slide-cell"-Effekt versagte, und, soviel ich weiss, wurde daselbst auf weitere Strahlenforschung verzichtet. Die Beweiskraft der Belege aus der Zeit, wo die „slide-cell"-Methode angeblich gute Resultate ergeben haben sollte, werden wir am Ende dieser Abhandlung beurteilen können.

Im Jahre 1932 und 1933 wurde im Laboratorium von Prof. Wolff (1932d, 1933b und e, 1934a) eine Zeitlang mit denselben Bakterienstämmen auch nephelometrisch gearbeitet (siehe Frank, 1932). In den letzteren beiden Publikationen wurden Daten angeführt, die beweisen sollten, dass die Trübung in dem Gebiet, in dem gearbeitet

[1]Siehe meine Originalarbeit, Westenberg (1935 Kap. 5).

wurde, sich ungefähr *linear* mit den „slide-cell" Zählungen ändere. In Anbetracht des Versagens des „slide-cell"-Effektes braucht man sich hinterher gar nicht zu wundern, dass auch die nephelometrische Methode wegen Misserfolgen ausser Gebrauch kam. Dies geschah allerdings schon im Jahre 1933 (Harders, S. 61, und mündliche Mitteilung von Prof. Wolff).

Die Streuung meiner Zahlen blieb, wie gesagt, trotz gehöriger Übung, viel grösser als die in den Protokollen von Frl. Ras, was man meiner ungenügenden Gewandtheit in der Arbeit zuschreiben wollte. Dieses nahm ich zum Anlass, die Fehlerquellen meiner Arbeitsweise eingehend zu studieren. Dabei musste ich die Aufmerksamkeit sowohl auf Faktoren, die den Bakteriengehalt der Suspensionsproben beeinflussen könnten, wie auf Messfehler, die eine Ungenauigkeit der abgemessenen Volumina verursachen wurden, richten.

Zunächst dachte ich an die Möglichkeit von Konzentrationsänderungen der Suspensionstropfen auf der Glasplatte durch Verdunstung, die bei kleinen Tropfen verhältnismässig grösser wären als bei grossen. Um diesem Übel vorzubeugen, wurden die mit Paraffin bestrichenen Platten während der Versuche in Glasdosen gelegt, in denen die Luft durch nasse Watte feucht gehalten war. Zum Benetzen der Watte gebrauchte ich Wasser, aber hinterher schien es, als wäre es doch richtiger gewesen, dazu irgend eine, mit der Flüssigkeit der Kokkensuspension isotonische Lösung zu benützen, da sonst das Wasser aus der Watte sich in den Suspensionstropfen kondensieren muss. Während der Arbeit ist es mir aber nicht aufgefallen, dass die Suspensionstropfen auf der Glasplatte grösser wurden; ich meine daher, dass diese Kondensation bei meinen Versuchsbedingungen nur in geringem Masse stattgefunden hat, während ohne die getroffene Vorsorge die Verdunstung manchmal ziemlich störend war.

Um zu entscheiden, ob die Suspension bei dem üblichen Verfahren der Probenentnahme wirklich genügend gemischt wurde, nahm ich zwei Versuchsserien vor, bei denen eine erhöhte Viskosität des Suspensionsmediums ein etwaiges Herabsinken der Kokken hindern sollte. Falls eine erhöhte Viskosität eine geringere Streuung der Keimzahlen ergäbe, würde das übliche Mischungsverfahren ungenügend sein. Dies war aber nicht der Fall, sodass keine Veranlassung mehr vorlag, nach weiteren Fehlerquellen zu suchen, die die Konzentration der Proben beeinflussen könnten.

Ferner schien es nötig zu prüfen, von welcher Grössenordnung die Fehler waren, die ich beim Abmessen der Probenvolumina machte.

Die höchste Genauigkeit des Abpipettierens wurde durch den Gebrauch einer Messpipette verbesserten Typs (Abb. 2b) erzielt, an welcher der Merkstrich durch eine Verengung ersetzt worden war. Saugt man die Suspension bis über diese Verengung auf, und drückt

danach das Gummihütchen wieder vorsichtig etwas zusammen, so bleibt der heruntergehende Meniskus knapp oberhalb der Verengung stehen. Nur das abzumessende Probenvolumen bleibt in der Pipette zurück, und lässt sich sodann durch etwas stärkeres Drücken auf das Gummihütchen in die "slide-cells" überführen.

Die gemachten Messfehler wurden durch serienweise Wägung von Proben bestimmt, die nach dieser Methode abgemessen waren. Diese erfolgte in einer feuchten Kammer mit einer Torsionswage in zwei Serien von je 20 Proben und einer Serie von 10 Proben. Die Standardabweichung der Einzelwägungen (von zirka 31 bis 34 mgr.) betrug in diesen drei Serien resp. 1.01%, 1.14% und 0.67%[2].

Die tatsächlich auftretende Streuung lässt sich keineswegs durch Messfehler dieser Grössenordnung erklären. Deshalb werden wir zunächst die Probenentnahme einer mathematischen Betrachtung unterziehen. Es wird sich nämlich herausstellen, dass man auch bei fehlerfreiem Arbeiten eine Streuung erwarten muss, da wir a priori die Eigenschaften dieser Streuung bestimmen können. In den experimentell erworbenen Zahlen wirkt die infolge der Messfehler entstandene und die a priori bestimmbare Streuung zusammen, wobei letztere den weitaus grössten Einfluss hat.

ZWEITER ABSCHNITT

Zur mathematischen Bearbeitung dieser Angelegenheit, nehmen wir zunächst die folgenden Grössen an;

Das Volumen der ganzen zu untersuchenden Suspension sei V. Die Gesamtzahl der darin vorhandenen Kokken sei N. Man denke sich diese Kokken numeriert von 1 bis N. Das Volumen jeder der abpipettierten Proben sei v. Die Anzahl von Kokken, die in jede der Proben gerät, nennen wir $n_1, n_2, n_3, n_4 \ldots . n_k$.

Denken wir uns nun das Volumen V restlos verteilt in eine bestimmte Anzahl Volumina v, so ist es ersichtlich, das wir $\frac{V}{v}$ Proben bekommen. Das arithmetische Mittel der Kokkenzahlen pro Probe ist somit

$$\tilde{n} = \frac{v}{V}.N. \quad [3] \qquad (1)$$

Wir werden uns nun mit der Frage beschäftigen, welche Anzahlen Kokken in den zu nehmenden Proben erwartet werden können, also welche Abweichungen von $\tilde{n}$ auftreten können. Zu diesem Zweck

[2]Für die bezügliche Protokolle sei auf meine Originalarbeit verwiesen (p. 25-26).

[3]Diese Ableitung trifft nur für den Fall zu, dass $\frac{V}{v}$ eine ganze Zahl ist. Für die Fälle, dass $\frac{V}{v}$ eine gebrochene oder eine unmessbare Zahl ist, lässt sich dieselbe Formel ableiten, wofür abermals auf meine Originalarbeit verwiesen sei (p. 28-30).

wollen wir für den Anfang nur *einen* bestimmten Kokkus c_1 ins Auge fassen; die anderen Kokken denken wir uns weg.

Wir können die vorhergehende Berechnung der mittleren Kokkenzahl pro Probe v auf diesen Fall anwenden, wobei nur dieser eine Kokkus c_1 vorhanden ist in V. Dadurch wird also $N = 1$ und unsere Formel

$$\tilde{n} = \frac{v}{V}.$$

Wir werden nun zeigen, dass $\frac{v}{V}$ auch genannt werden kann: „Die Wahrscheinlichkeit, mit welcher c_1 in einer bestimmten Probe v angetroffen wird", denn die Definition von „Wahrscheinlichkeit" lautet in diesem Falle: Der Limes eines Verhältnisses, und zwar hier des Verhältnisses der Anzahl Proben, in denen c_1 angetroffen wird, zur Gesamtzahl der zu nehmenden Proben, wobei jedes Volumelement von V auf gleiche Weise in die Berechnung einbezogen wird.

In dem Fall, in dem nur ein Kokkus vorhanden ist, beträgt die „Anzahl Kokken" pro Probe v, entweder 1 oder 0. Ob wir nun daran gehen, das arithmetische Mittel all dieser „Anzahlen" zu bestimmen oder ob wir die Wahrscheinlichkeit für das Antreffen von c_1 berechnen wollen, in beiden Fällen müssen wir die Anzahl der Fälle notieren, in denen wir c_1 antreffen, und diese Anzahl durch die Gesamtzahl der bearbeiteten Proben teilen.

Die Wahrscheinlichkeit p für das Auftreten eines bestimmten Kokkus in einer bestimmten Probe v ist also gleich dem arithmetischen Mittel wenn $N = 1$, also

$$\mathrm{p} = \frac{v}{V} \tag{2}$$

Die Wahrscheinlichkeit für das Nicht-Vorhandensein eines bestimmten Kokkus in einer bestimmten Probenmenge ist

$$q = 1 - p \tag{3}$$

Wir dehnen nun unsere Betrachtungen aus auf alle N Kokken, die wir uns, wie schon gesagt, nummeriert denken, wobei jedoch die Verteilung der Nummern bei den Kokken willkürlich ist.

Als eine beliebige n-Zahl aus diesen N Kokken können wir also ansehen: $c_1, c_2, c_3, \ldots, c_n$.

Die Wahrscheinlichkeit, dass irgendein zu nehmendes Probenvolumen diese n-Zahl enthält, ist p^n, was sich folgendermassen nachweisen lässt.

Die Wahrscheinlichkeit, dass c_1 vorhanden ist, beträgt p. Das bedeutet, dass das Verhältnis der Anzahl c_1-enthaltender Proben zur Gesamtzahl der entnommenen Proben $p:1$ ist.

Hält man nun das Auftreten von c_2 *für unabhängig vom Auftreten von* c_1, dann läuft das darauf hinaus, dass das Verhältnis der Anzahl

$c_1 + c_2$ enthaltender Proben sich wiederum zur Anzahl c_1-enthaltender Proben verhält wie $p:1$, also zur Gesamtzahl der genommenen Proben wie $p^2:1$, mit andern Worten die Wahrscheinlichkeit, dass eine gewisse Probe sowohl c_1 wie c_2 enthält ist p^2.

So fortfahrend findet man für die Wahrscheinlichkeit, dass eine gewisse Probe $c_1 + c_2 + c_3 \ldots c_n$ enthält, p^n.

Auf ähnliche Weise findet man für die Wahrscheinlichkeit, dass ein gewisses Volumen v *nur* $c_1 + c_2 + c_3 \ldots c_n$, aber die übrigbleibenden $N—n$ Kokken *nicht* enthält, $p^n.q^{N-n}$.

Das Ergebnis von n Kokken in unserer Probe bekommt man jedoch auch, wenn eine andere n-Zahl gewählt wird. Es lässt sich nämlich aus den vorhandenen N Kokken in vieler Weise eine Gruppe von n Kokken bilden. Der möglichen Kombinationen gibt es

$$C_N^n = \frac{N!}{n!(N—n)!} \quad *) \tag{4}$$

Für die Wahrscheinlichkeit, dass eine Probe irgendwie n Kokken enthält, kommen wir also zu der durchaus bekannten Formel

$$P_{(n)} = C_N^n \; p^n \, q^{N-n} = \frac{N!}{n!(N—n)!} \, p^n \, q^{N-n} \tag{5}$$

Bei festem N, festem p, (also auch festem q) hat diese Wahrscheinlichkeit also für jedes n einen bestimmten Wert.

Wir werden uns nun mit den Abweichungen beschäftigen, die n von $\tilde{n}$ zeigen kann.

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*Denken wir uns alle C_N^n Kombinationen der Kokken c_1, c_2, $c_3 \ldots c_N$ aufgeschrieben.

Jetzt zählen wir die Kombinationen mit c_1 darin, und auch die mit den anderen c's damit alle Individuen gleichwertig sind; die Zahl ist C_{N-1}^{n-1}; für die N Kokken zählen wir $N \,.\, C_{N-1}^{n-1}$; aber jetzt ist jede Kombination, weil diese n Individuen enthält, auch n mal gezählt worden.

Wir finden also

$$C_N^n = \frac{N}{n} \,.\, C_{N-1}^{n-1}$$
$$C_{N-1}^{n-1} = \frac{N-1}{n-1} \; C_{N-2}^{n-2}$$
$$\ldots\ldots\ldots\ldots$$
$$C_{N-(n-1)}^{1} = N-(n-1)$$

Multiplikation ergibt:

$$C_N^n = \frac{N \,.\, (N—1) \ldots ((N—(n—1))}{n \,.\, (n-1) \ldots 2 \,.\, 1} = \frac{N!}{n!(N—n)!}$$

Die Abweichung, die ein gewisser Wert von n gegen $\widetilde{n}$ hat, setzen wir gleich u, sodass

$$u = n - \widetilde{n} = n - p.N. \tag{6}$$

Das Quadrat dieser Abweichung schreiben wir u^2 und der durchschnittliche Wert von u^2 einer Serie sei $\overline{u^2}$.

Die Wahrscheinlichkeit eines gewissen Wertes von n war $P_{(n)}$, und der Natur der Sache nach, ist dies zugleich die Wahrscheinlichkeit des zugehörigen Wertes von u.

Ist nun f die Frequenz eines gewissen Wertes von u, dann ist

$$\overline{u^2} = \frac{\Sigma u^2}{k} = \frac{f_1.u_1^2 + f_2 u_2^2 + f_3.u_3^2 + \cdots}{k} \tag{7}$$

wobei dann $\Sigma f = f_1 + f_2 + f_3 + f_4 \ldots = k$.

Wir werden nun versuchen abzuleiten, welchen Wert (7) annimmt, wenn wir voraussetzen, dass die Frequenzen sich verhalten wie ihre resp. Wahrscheinlichkeiten, m.a.W. $f_1 = P_{(n_1)} . k$; $f_2 = P_{(n_2)} . k$ u.s.w., oder

$$\frac{f_1}{P_{(n_1)}} = \frac{f_2}{P_{(n_2)}} = \frac{f_3}{P_{(n_3)}} = \cdots = k \tag{8}$$

Diese Grösse, nämlich ein $\overline{u^2}$, das zu Frequenzen gehört, die mit (8) übereinstimmen, nennen wir die „mathematische Erwartung von u^2", geschrieben $\widetilde{u^2}$. Wenn jedoch (8) genügt ist, können allerlei $f's$ einen Bruchwert annehmen, wodurch die gesuchte Grösse $\widetilde{u^2}$ ihre reelle Bedeutung verliert. Durch die folgenden Überlegungen können wir ihr gleichwohl eine Bedeutung beilegen.

Die Keimzahlen einer Serie von k Proben werden gewöhnlich der Voraussetzung (8) nicht genügen. Wir dehnen unsere Betrachtungen deshalb auf mehrere Serien aus, von k Proben von Suspensionen mit gleichem $\widetilde{n}$. Je mehr Serien wir in Anschlag bringen, umso mehr wird sich das Verhältnis der Frequenzen der verschiedenen Werte von n im gesamten Zahlenmaterial dem Verhältnis der resp. Wahrscheinlichkeiten $P_{(n)}$ nähern.

Tatsächlich ist diese Ausführung nichts anderes als eine Wiederholung der Wahrscheinlichkeits-Definition, und zwar der Wahrscheinlichkeit $P_{(n)}$.

Hat in jeder Serie von k Proben die Grösse $\overline{u^2}$ einen gewissen Wert, dann stellt also $\widetilde{u^2}$ den durchschnittlichen Wert von u^2 vor, aus unendlich vielen Serien aus Suspensionen mit gleichem $\widetilde{n}$.

Nachdem wir also $\widetilde{u^2}$ eine Bedeutung gegeben haben, beginnen wir nun mit der Ableitung seines Wertes.

$$\widetilde{u^2} = \frac{f_1 \cdot u_1^2 + f_2 \cdot u_2^2 + f_3 \cdot u_3^2 + \cdots}{\Sigma f}$$

Ist der Voraussetzung für (8) genügt, dann kann man dafür setzen:

$$\frac{P_{(n\,1)} \cdot u_1^2 + P_{(n\,2)} \cdot u_2^2 + P_{(n\,3)} \cdot u_3^2 + \cdots}{\Sigma P_{(n)}} =$$

$$P_{(n\,1)} \cdot u_1^2 + P_{(n\,2)} \cdot u_2^2 + P_{(n\,3)} \cdot u_3^2 + \cdots =$$

$$\sum_{u\,=\,0-pN}^{u\,=\,N-pN} P_{(n)} \cdot u^2 = \sum_{n\,=\,0}^{n\,=\,N} P_{(n)} \cdot (n-pN)^2 =$$

$$\sum_{n\,=\,0}^{n\,=\,N} P_{(n)} \cdot n^2 - 2 \sum_{n\,=\,0}^{n\,=\,N} P_{(n)} \cdot n \cdot pN + \sum_{n\,=\,0}^{n\,=\,N} P_{(n)} \cdot (pN)^2.$$

Dieses schreiben wir: $A + B + C$.

$$A = \sum_{n\,=\,0}^{n\,=\,N} P_{(n)} \cdot n^2 =$$

$$\sum_{n\,=\,0}^{n\,=\,N} P_{(n)} \cdot n \cdot (n-1) + \sum_{n\,=\,0}^{n\,=\,N} P_{(n)} \cdot n =$$

$$\sum_{n\,=\,0}^{n\,=\,N} n \cdot (n-1) \cdot \frac{N!}{n!(N-n)!} \cdot p^n \cdot q^{N-n} + p \cdot N$$

Da die Ausdrücke n und $(n-1)$ vorhanden sind, lässt sich hierfür schreiben:

$$\sum_{n\,=\,2}^{n\,=\,N} n \cdot (n-1) \cdot \frac{N!}{n!(N-n)!} \cdot p^n \cdot q^{N-n} + p \cdot N =$$

$$N(N-1) \cdot p^2 \sum_{n\,=\,2}^{n\,=\,N} \frac{(N-2)!}{(n-2)!((N-2)-(n-2))!} p^{n-2} \cdot q^{((N-2)-(n-2))} + p \cdot N$$

Wen n von 2 bis N geht, dann geht $(n-2)$ von 0 bis $(N-2)$. Setzen wir nun $(n-2) = \Omega$:

$$N \cdot (N-1) \cdot p^2 \sum_{\Omega=0}^{\Omega=N-2} \frac{(N-2)!}{\Omega!((N-2)-\Omega)!} \cdot p^{\Omega} \cdot q^{(N-2)-\Omega} + p \cdot N =$$

$$N \cdot (N-1) \cdot p^2 \cdot (p+q)^{N-2} + p \cdot N =$$

$$N \cdot (N-1) \cdot p^2 \cdot 1 + p \cdot N =$$

$$p^2 N^2 - p^2 N + p N \cdot$$

$$B = -2 \sum_{n=0}^{n=N} P_{(n)} \cdot n \cdot p N = -2 p N \sum_{n=0}^{n=N} P_{(n)} \cdot n =$$

$$-2 p N \cdot p N = -2 p^2 N^2 \cdot$$

$$C = \sum_{n=0}^{n=N} P_{(n)} \cdot (p N)^2 = (p N)^2 \sum_{n=0}^{n=N} P_{(n)} =$$

$$(p N)^2 . 1 = p^2 N^2.$$

Zusammenfassend ergibt das:

$$\widetilde{u^2} = A + B + C =$$

$$p^2 N^2 - p^2 N + p N - 2 p^2 N^2 + p^2 N^2 =$$

$$p(1-p) N = p q N. \qquad (9)$$

Schliesslich führen wir eine kleine Annäherung ein, indem wir $q = 1$ stellen. In unseren Versuchen ist p klein, denn v ist von der Grössenordnung 0,05 cm³ und V von der Ordnung 10 cm³. Die Annäherung $q = 1$ hat also keine grosse Ungenauigkeit zur Folge. So kommen wir zu:

$$\widetilde{u^2} = \widetilde{n} \qquad (10)$$

Diese Aussage in Bezug auf die zu erwartende Zahlenstreuung lässt sich jedoch nicht ohne weiteres empirisch nachprüfen, weil bei den Versuchen $\widetilde{n}$ und also auch u unzugänglich sind. Wir werden uns deshalb bemühen, obige Beziehung (10) in eine andere umzuformen in Werten, die wohl zugänglich sind. Dazu werden wir statt $\widetilde{n}$, das zugängliche $\overline{n}$, nebst den Abweichungen der Einzelzählungen gegen diese Grösse, in die Formel einzuführen.

Die Abweichung die ein $\overline{n}$ gegen $\widetilde{n}$ zeigt, setzen wir gleich v, sodass $v = \overline{n} - \widetilde{n}$.

Für jede Probenserie gilt:

$$\Sigma u^2 = \Sigma(n - \tilde{n})^2 =$$

$$\Sigma (n - \bar{n} + \bar{n} - \tilde{n})^2 =$$

$$\Sigma (x + v)^2 =$$

$$\Sigma x^2 + 2\,\Sigma x.v + \Sigma v^2 =$$

$$\Sigma x^2 + 2v\,\Sigma x + k.v^2 =$$

$$\Sigma x^2 + 0 + k.v^2. \qquad (11)$$

Nun ist

$$v = \bar{n} - \tilde{n} = \frac{(n_1 - \tilde{n}) + (n_2 - \tilde{n}) + \cdots + (n_k - \tilde{n})}{k} =$$

$$\frac{u_1 + u_2 + u_3 + \cdots + u_i + \cdots + u_j + \cdots + u_k}{k}.$$

und also ist

$$v^2 = \frac{\Sigma u^2 + 2\,\Sigma u_i . u_j}{k^2}$$

Durch Substition dieses Wertes in Formel (11) kommt man zu:

$$\Sigma u^2 = \Sigma x^2 + \frac{\Sigma u^2}{k} + \frac{2\,\Sigma u_i . u_j}{k}$$

Folglich schreiben wir:

$$k \cdot \overline{x^2} = \Sigma x^2 = \frac{k \cdot \Sigma u^2}{k} - \frac{\Sigma u^2}{k} - \frac{2\Sigma u_i \cdot u_j}{k} = \frac{k-1}{k} \cdot \Sigma u^2 - \frac{2\Sigma u_i \cdot u_j}{k}$$

Da die Abweichungen u von $\tilde{n}$ einmal positiv, ein andermal negativ sind, werden wir $2\Sigma u_i \cdot u_j$ vernachlässigen können gegen $(k-1) \cdot \Sigma u^2$, zumal wenn wir bald unsere Betrachtung über unendlich viele Serien ausdehnen werden.

Wir schreiben also:

$$\frac{k}{k-1} \cdot \frac{\Sigma x^2}{k} = \frac{\Sigma u^2}{k}$$

Für jede Serie schwankt dieser Wert um $\tilde{n}$.

Betrachten wir nun eine Gruppe von unendlich vielen Serien mit demselben $\bar{n}$, so schwankt dieser Wert um $\bar{n}$.

Unter der mathematischen Erwartung von x^2, geschrieben $\tilde{x^2}$, verstehen wir den durchschnittlichen Wert von x^2 in unendlich vielen Serien mit demselben $\bar{n}$. Also

$$\widetilde{x^2} = \frac{k-1}{k} \cdot \overline{n} \qquad (12)$$

Es ist klar, dass diese Aussage in Bezug auf die zu erwartende Zahlenstreuung (im Gegensatz zu Formel (10)), eine empirische Nachprüfung gestattet, da bei den Versuchen x, $\overline{n}$ sowie k zugänglich sind.

Für verschiedene Werte von k ist diese Beziehung in Abb. 4 graphisch dargestellt. Da ich bei meiner Arbeit keine grossen Fehlerquellen entdecken konnte, lässt sich erwarten, dass das $\overline{x^2}$ in meinen Serien annähernd um diese mathematische Erwartung schwanken wird, die wir gerade ableiteten. In Abb. 5 und 6 sehen wir, wie unsere Serien von 4 und von 16 Zahlen sich dieser mathematischen Erwartung gegenüber verhalten: $\overline{n}$ steht hierbei an der horizontalen, und $\overline{x^2}$ jeder Serie an der vertikalen Achse. Tatsächlich fallen die Punkte, wie zu erwarten war zu beiden Seiten der Linie, die $\widetilde{x^2}$ als Funktion von $\overline{n}$

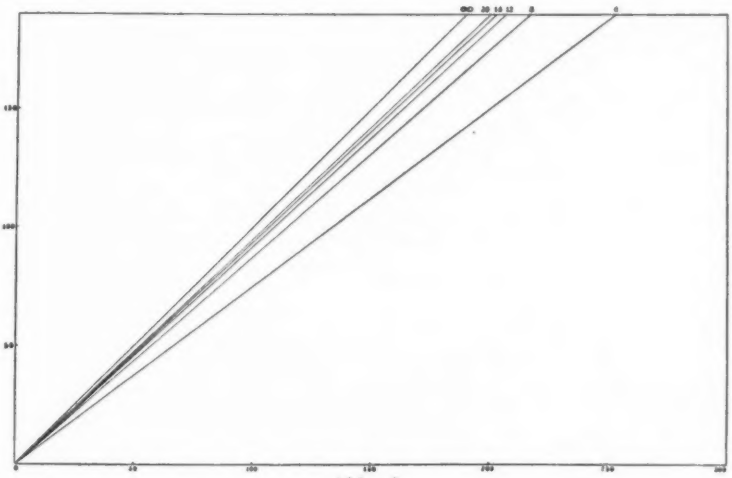

Abb. 4

wiedergibt. Wohl fallen mehr Punkte unterhalb als oberhalb der Linie, was bei den Viererserien auffälliger ist als bei den Serien von je 16 Zahlen. Diese Tatsache fordert weiterhin nähere Beachtung.

Wollen wir die Streuung in den Ras' schen Protokollen auf dieselbe Weise studieren, so stossen wir zuerst auf die Schwierigkeit, dass in den Publikationen fast immer nur die Durchschnitte der Serien angegeben werden, sodass sich hier die ursprüngliche Zahlenstreuung bei dieser Notierung nicht studieren lässt.

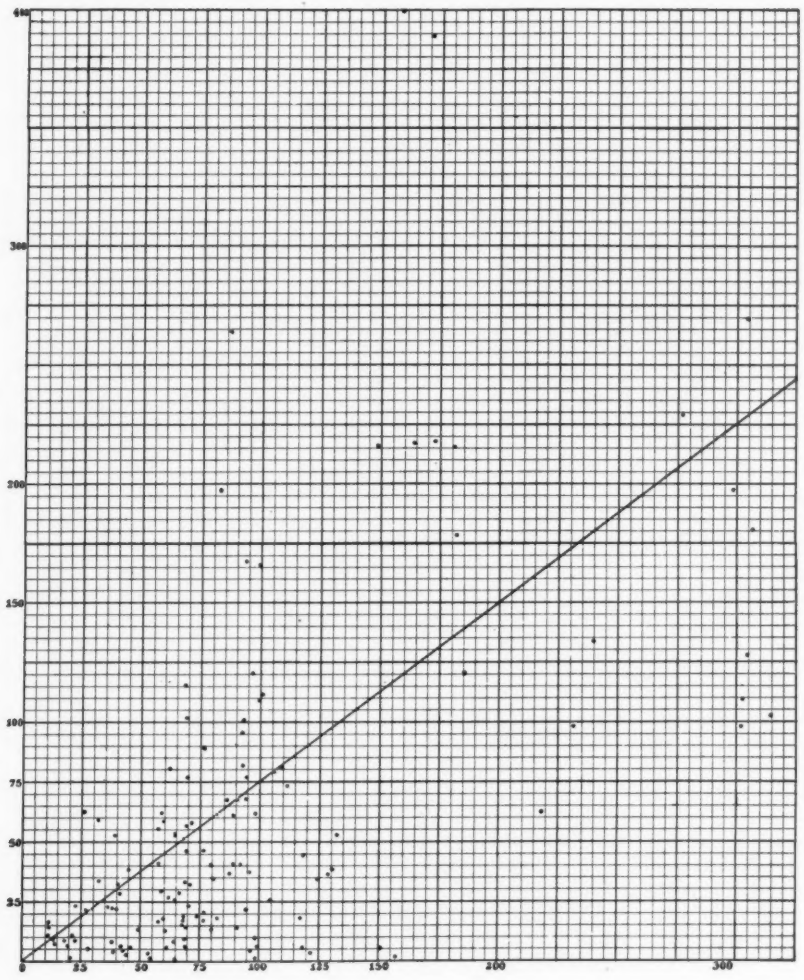

Abb. 5

Die spärlichen vollständigen Angaben, die sich in den Publikationen von Wolff und Ras vorfinden, werden wir zunächst zusammenstellen; so zitieren wir aus der Veröffentlichung (1932e) S. 260 „Um eine Vorstellung von dem Werte und den Fehlern dieser Methode zu vermitteln, teilen wir hier einige Daten mit:"

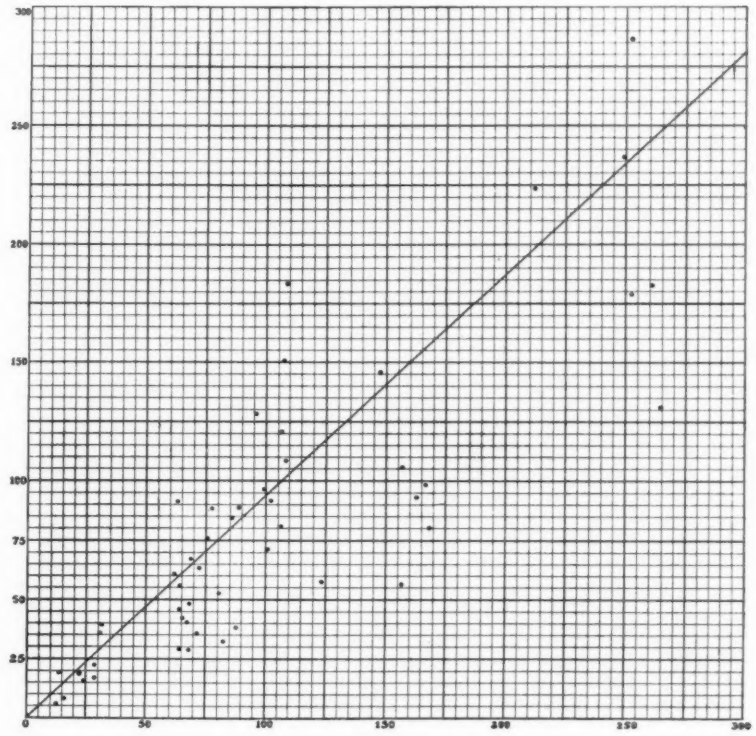
300
250
200
150
100
75
50
25
0
50
100
150
200
250
300

Abb. 6

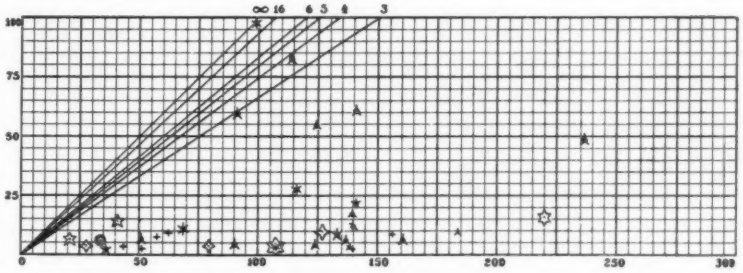
∞ 16 6 5 4 3
100
75
50
25
0
50
100
150
200
250
300

Abb. 7

	Einzelzählungen n						Mittelwert $\overline{n}$	Mittleres Quadrat der Abweichungen vom Mittelwert. $\overline{x^2}$
	A Agarkulturen							
I	18	19	20	21	25		20.6	5.84
II	36	39	40	42	47		40.8	13.36
III	104	106	107	109	109	110	107.5	4.25
IV	215	217	218	220	223	227	220.0	16.0
	B Slide-cells							
I	25	27	28	30			27.5	3.25
II	77	78	80	82			79.3	3.52
III	123	125	129	131			127.0	10.0

In Abb. 7 finden wir das $\overline{x^2}$ von jeder dieser Serien graphisch mittels der grossen Sterne ✧ dargestellt. Die Anzahl der Strahlen jedes Sternes gibt an, aus wieviel Zahlen die betreffende Serie besteht; von dieser Anzahl hängt es ab, mit welcher mathematischen Erwartung die graphisch dargestellte Streuung verglichen werden muss.

Diese Sterne in Abb. 7 scheinen nun nicht im mindesten den theoretisch abgeleiteten Erwartungen zu entsprechen. Es geht natürlich nicht an, die Arbeit von Jahren nach diesen sieben Serien beurteilen zu wollen, aber durch das grosse Entgegenkommen von Prof. Wolff konnte ich mir einen Eindruck bilden von der durchschnittlichen Streuung bei den „slide-cell"-Untersuchungen in seinem Laboratorium. So fand ich, dass die sieben angeführten Serien wirklich ein richtiges Bild der Streuung in den Ras' schen Protokollen geben.

Auch das nachfolgende Zahlenmaterial, das wir noch den Veröffentlichungen entnehmen, stimmt im Grossen und Ganzen damit überein:

Das $\overline{x^2}$ der oberen Serien ist in der graphischen Darstellung durch Sterne des Typus ✲ angegeben, und für die letzte durch +, wobei die Anzahl Strahlen der Sterne die Anzahl Zahlen der Serie (also k) anzeigt[6].

Im ganzen kann man sagen, dass die Streuung diese Zahlenmaterials weit unternormal ist. Mathematisch gesehen ist es auch sonderbar, dass das $\overline{x^2}$ hier keine deutliche Abhängigkeit von $\overline{n}$ zeigt, und offenbar nicht den Gesetzen des Zufalls folgt.

Da das zitierte Zahlenmaterial aus den Veröffentlichungen von Wolff und Ras doch immer noch spärlich ist, werden wir versuchen, die Streuung auch noch von einem anderen Kriterium aus zu prüfen. Es liegen nämlich Angaben über den Wert des „Messungsfehlers".

[6] Bei Raummangel ✦ anstatt ✧ u. s. w.

oder kurz des „Fehlers" der Methode vor, womit die Standardabweichung der Einzelzählungen gemeint wird, also $S_{(n)} = \sqrt{\frac{\Sigma x^2}{k-1}}$ (persönliche Mitteilung von Prof. Wolff).

Entnimmt man einer Suspension einige Serien von Proben, und berechnet für jede Serie $S_{(n)} = \sqrt{\frac{\Sigma x^2}{k-1}}$, dann wird man gewöhnlich für

Wolff und Ras (1932e) p. 261

Einzelzählungen n						Mittelwert $\overline{n}$	Mittleres Quadrat der Abweichungen vom Mittelwert. $\overline{x^2}$
133	139	142	145	146		141	22
135	139	145				139.7	16.85
88	90	93	99	110	114	99	97.33
132	140	151				141	60.66
198	209	244				217	384.66 [5]
109	113	113	118	120	125	116.3	27.88
106	110	127				114.3	82.88
118	121	135				124.7	54.88
Wolff und Ras (1932e) p. 262							
34	34	35	36	37		35.2	1.36
47	52	53				50.7	6.88
81	95	99				91.7	59.55
64	66	67	70	70	74	68.5	10.58
88	90	93				90.3	4.22
134	137	140				137	6
104	106	107	109	109	110	107.5	4.25
122	124	127				124.3	4.22
129	130	135	135	136		133	8.4
157	161	163				160.3	6.22
227	240	243				236.7	48.22
Wolff und Ras (1933d) p. 306							
29	36	32	36			33.7	5.84
33	35	36	33				
32	37	31	35				
36	36	30	32				
60	58	53	59			57.5	7.25
42	42	45	46			43.8	3.19
66	60	59	65			62.5	9.25
53	52	49	50			51	2.5
139	140	142	138			139.8	2.19
179	186	185				183.3	9.55
159	155	152	159			156.3	8.69
139	140	136				138.3	2.88
145	137	138				140	12.66
137	145	139				140.3	11.55

[5] Liegt ausserhalb der graphischen Darstellung.

jede Serie einen anderen Wert von $S_{(n)}$ finden. Dieses $S_{(n)}$ ist also eine variable Grösse. Wir werden uns nun bemühen, die mathematische Erwartung dieser Grösse zu ermitteln, da diese uns einen Halt zum Vergleich der bezüglichen Angaben darbieten wird.

Wie wir bei der Ableitung von $\widetilde{u}^2$ sahen, lässt sich die mathematische Erwartung einer variablen Grösse ausrechnen durch das Summieren aller Produkte: Wert × Wahrscheinlichkeit dieses Wertes. Aus mathematischtechnischen Gründen lässt sich diese Addition am besten kontinuierlich ausführen, sodass es nun ankommt auf die Lösung von

$$\widetilde{S}_{(n)} = \int_0^\infty S_{(n)} \cdot d\,f$$

Die Wahrscheinlichkeit eines gewissen Wertes von S wurde von dem englischen Statistiker R. A. Fisher (1925) studiert für den Fall, dass n der Gausschen Frequenzverteilung folgt. Wenn aber $\widetilde{n}$ unweit 100, wie es unseren Versuchsverhältnissen entspricht, so nähert sich unsere binomiale Frequenzverteilung der Gauss' schen bekanntlich schon derart, dass wir ohne weiteres die Fishersche Formel anwenden dürfen. Also ist die Wahrscheinlichkeit, dass das Quadrat einer Standardabweichung in das Interval $(S^2, S^2 + d(S^2))$ fällt, gleich

$$d\,f = \frac{(k-1)^{\frac{k-1}{2}}}{2^{\frac{k-1}{2}} \cdot \Gamma\left(\frac{k-1}{2}\right)} \cdot \frac{S^{k-3}}{\sigma^{k-1}} \cdot e^{-\frac{(k-1)\,.\,S^2}{2\,.\,\sigma^2}} \cdot d\,(S^2) \cdot \qquad (13)$$

Hier steht σ^2 für $\widetilde{S}^2$ und folglich σ für $\sqrt{\widetilde{S}^2}$.

Für die Ableitung dieses Gesetzes sei auf Fisher verwiesen.

Da nun die Wahrscheinlichkeit irgend eines Wertes von S ebenso gross ist als die des zugehörigen Wertes von S^2, lässt sich $\widetilde{S}$ berechnen aus:

$$\widetilde{S} = \int_0^\infty S \cdot d\,f =$$

$$\frac{(k-1)^{\frac{k-1}{2}}}{2^{\frac{k-1}{2}} \cdot \Gamma\left(\frac{k-1}{2}\right)} \cdot \int_{S^2=0}^{S^2=\infty} S \cdot \frac{S^{k-3}}{\sigma^{k-1}} \cdot e^{-\frac{(k-1)\,.\,S^2}{2\,.\,\sigma^2}} \cdot \mathrm{d}\,(S^2) =$$

$$\frac{\left(\frac{k-1}{2}\right)^{\frac{k-1}{2}}}{\Gamma\left(\frac{k-1}{2}\right)} \cdot \int_{S=0}^{S=\infty} S \cdot \frac{S^{k-3}}{\sigma^{k-1}} \cdot e^{-\frac{(k-1)\,.\,S^2}{2\,.\,\sigma^2}} \cdot 2\,S \cdot d\,S =$$

$$2 \cdot \frac{\left(\frac{k-1}{2}\right)^{\frac{k-1}{2}}}{\Gamma\left(\frac{k-1}{2}\right)} \cdot \int_{S=0}^{S=\infty} \left(\frac{S}{\sigma}\right)^{k-1} \cdot e^{-\left(\frac{k-1}{2}\right) \cdot \left(\frac{S}{\sigma}\right)^2} \cdot d\,S =$$

$$2\,\sigma \cdot \frac{\left(\frac{k-1}{2}\right)^{\frac{k-1}{2}}}{\Gamma\left(\frac{k-1}{2}\right)} \cdot \int_{\frac{S}{\sigma}=0}^{\frac{S}{\sigma}=\infty} \left(\frac{S}{\sigma}\right)^{k-1} \cdot e^{-\left(\frac{k-1}{2}\right) \cdot \left(\frac{S}{\sigma}\right)^2} \cdot d\left(\frac{S}{\sigma}\right).$$

Diese Darstellung lässt sich dadurch vereinfachen, dass man

für $\frac{S}{\sigma}$ die neue Variable ζ und

für $\frac{k-1}{2}$ den Buchstaben ρ einführt; alsdann ergibt sich

$$2\,\sigma \cdot \frac{\rho^\rho}{\Gamma(\rho)} \cdot \int_{\zeta=0}^{\zeta=\infty} \zeta^{2\rho} \cdot e^{-\rho \cdot \zeta^2} \cdot d\,\zeta.$$

Man führt nun abermals eine neue Variable ein, nämlich ξ, die definiert wird durch $\xi^{\frac{1}{2}} = \rho^{\frac{1}{2}} \,.\, \zeta$, sodass $\zeta = \rho^{-\frac{1}{2}} \,.\, \xi^{\frac{1}{2}}$;

also $\zeta^2 = \rho^{-1} \,.\, \xi$ und $\zeta^{2\rho} = \rho^{-\rho} \,.\, \xi^\rho$ und $d\,\zeta = \rho^{-\frac{1}{2}} \,.\, \frac{1}{2} \,.\, \xi^{-\frac{1}{2}} \,.\, d\,\xi$.

Die obige Formel verwandelt sich dann in:

$$2\,\sigma \cdot \frac{\rho^\rho}{\Gamma(\rho)} \cdot \int_{\xi=0}^{\xi=\infty} \rho^{-\rho} \cdot \xi^\rho \cdot e^{-\zeta} \cdot \frac{1}{2} \cdot \rho^{-\frac{1}{2}} \cdot \xi^{-\frac{1}{2}} \cdot d\,\xi =$$

$$2\,\sigma \cdot \frac{\rho^\rho}{\Gamma(\rho)} \cdot \rho^{-\rho} \cdot \frac{1}{2} \cdot \rho^{-\frac{1}{2}} \int_{\xi=0}^{\xi=\infty} \xi^{\rho-\frac{1}{2}} \cdot e^{-\xi} \cdot d\,\xi =$$

$$\frac{\sigma}{\varrho^{\frac{1}{2}} \cdot \Gamma(\varrho)} \int_{\xi=0}^{\xi=\infty} \xi^{(\varrho+\frac{1}{2})-1} \cdot e^{-\xi} \cdot d\xi =$$

$$\frac{\sigma}{\varrho^{\frac{1}{2}} \cdot \Gamma(\varrho)} \cdot \Gamma(\varrho + \tfrac{1}{2}) =$$

$$\sigma \cdot \frac{\Gamma(\varrho + \frac{1}{2})}{\varrho^{\frac{1}{2}} \cdot \Gamma(\varrho)}.$$

Schliesslich schreibt man:

$$\widetilde{S}_{(n)} = \sqrt{\widetilde{S}^2_{(n)}} \cdot \sqrt{\frac{2}{k-1}} \cdot \frac{\Gamma(\frac{1}{2}k)}{\Gamma(\frac{1}{2}k-\frac{1}{2})} \qquad (14)$$

Eine einfache Erwägung lehrt, dass

$$\widetilde{S}^2_{(n)} = \widetilde{w}^2 = \widetilde{n}$$

also wird

$$\widetilde{S}_{(n)} = \sqrt{\widetilde{n}} \cdot \sqrt{\frac{2}{k-1}} \cdot \frac{\Gamma(\frac{1}{2}k)}{\Gamma(\frac{1}{2}k-\frac{1}{2})} \qquad (15)$$

Die Anzahl Proben pro Serie war in der ersten Veröffentlichung recht verschieden, aber bald wurde es zur festen Regel, fast nur mit Serien von 4 Zählungen zu arbeiten. So war es auch üblich, als ich mich im Sommer 1933 im Utrechter Laboratorium in die Methode einübte, und so wurde es wenigstens bis 1 Juni 1934 durchgeführt, wo ich Prof. Wolff darauf aufmerksam machte, das 4 Zählungen (unweit 100) pro Serie zum Nachweis einer Bakterienvermehrung von 20% nicht genügen.[7] Der weitaus grösste Teil der Experimente, auf die sich die letzte Publikation (1934b) gründet, war bereits vordem abgeschlossen, sodass also so gut wie alle Angaben betreffs der Standardabweichung sich beurteilen lassen unter Annahme $k = 4$, wobei sich die Formel (15) verwandelt in:

$$S_{(n)} = 0{,}614 \ldots \sqrt{\widetilde{n}}. \qquad (16)$$

In Abbildung 8 wird diese Funktion graphisch dargestellt und wir sehen, dass ihr Wert bei $\widetilde{n} = 150{,}8 \ldots 5\%$ von $\widetilde{n}$ beträgt, d. h. dass wir bei $\widetilde{n} = 150$ noch ungefähr in der Hälfte der Fälle eine Standardabweichung erwarten mögen, die 5% von n überschreitet.

Mit den Angaben hinsichtlich der üblichsten Werte von $\widetilde{n}$ ist es weniger einfach bestellt, was durchaus verständlich ist, da man es nicht in der Hand hat, diese Grösse von vornherein genau zu bestimmen.

[7] Siehe meine Originalarbeit, Kap. 6.

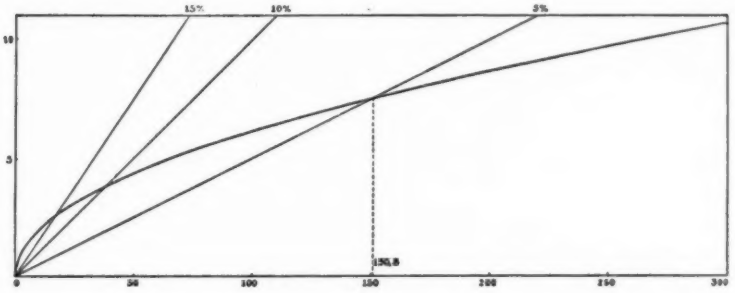

Abb. 8

Anfangs (Wolff und Ras, 1931) lagen die Grenzen ziemlich weit auseinander, nämlich 10-200. Bei zunehmender Gewandtheit wurden diese Grenzen enger genommen, sodass zirka vom Juli 1933 an 60-80 als meist erwünschte Zahl angesehen wurde (mündliche Mitteilung). Weniger bestimmt sind die diesbezüglichen Angaben wieder in der letzten Publikation (1934b), die wie gesagt, kurz nach unserer Unterhaltung erschien; auf Seite 430 lesen wir 40-200, auf Seite 432 dagegen 50-150.

Aus alledem möchte ich schliessen, dass unsere Abschätzung eher zu gross als zu klein sein wird, wenn wir annehmen, dass die Keimzahl bei den Versuchen von Frl. Ras meistens, oder doch wenigstens sehr oft zwischen 80 und 100 gelegen habe.

Diese Ausführungen ermöglichen nun die Beurteilung der nachfolgenden Angaben:

Wolff und Ras	1931	S. 137	Messungsfehler	höchstens	10—15 %
" " "	1931e	S. 270	"	nicht grösser als	10—15 %
" " "	1931f	S. 280	"	" " "	10—15 %
Wolff	1932	S. 3285	"	" " "	5—10 %
Wolff und Ras	1932d	S. 144	"	höchstens	5 %
" " "	1934a	S. 594	"	"	10 %
" " "	1933b	S. 875	"	"	5 %
" " "	1933e	S. 315	"	"	5 %
" " "	1934b	S. 431	"	gewöhnlich nicht grösser als	5 %

(„erreur expérimentale ne dépasse pas en moyenne 5 %")

Es ist klar, dass diese Angaben mit einer mathematischen Erwartung von 5% bei $\tilde{n} = 150{,}8$ nicht vereinbar sind. Wir kommen also auch nach diesem Kriterium zu dem Schluss, dass die Streuung in den Ras' schen Protokollen bedeutend kleiner ist, als man nach den vorgeführten aprioristischen Betrachtungen erwarten möchte.

DRITTER ABSCHNITT

Wir werden nun überlegen, welche Ursachen zur Aufklärung dieses Umstandes in Betracht kommen. Man könnte sich nämlich denken, dass die Voraussetzungen unserer Ableitungen den Versuchsverhältnissen nicht genügend entsprächen, auch könnte irgend eine Täuschung vorliegen. Wir werden versuchen, alle Möglichkeiten erschöpfend darzustellen.

Erstens könnten die Kokken in der Suspension gegenseitig ihre Lage in merklicher Weise beeinflussen, während bei der Ableitung der Formeln vorausgesetzt wurde, dass dem nicht so sei. Es ist dies tatsächlich der Fall, denn jeder Kokkus steht in dem Teil des Raumes, den er selbst einnimmt, jedem andern im Wege. Hierdurch wird die Streuung etwas kleiner werden als sie es wäre, wenn wir mit mathematischen Punkten zu tun hätten. Da wir aber das Gesamtvolumen der Kokken auf weniger als ein Millionstel der ganzen Suspension schätzen, lässt sich dieser Faktor ruhig vernachlässigen.

Zweitens könnten die Kokken auf andere Weise ihre Lage gegenseitig beeinflussen; es wäre nämlich denkbar, dass die Kokken in dem Suspensions-Medium sich gegenseitig anziehen oder abstossen. Im letzteren Falle würden die Kokken danach streben, sich wenigstens im Innern der Suspension (von wo wir die Proben entnehmen) wie ein Raumgitter zu verteilen, was eine beträchtlich geringere Zahlenstreuung zur Folge haben würde.

Berücksichtigen wir, dass die Zeit zwischen dem Mischen und der Probenentnahme bei den Versuchen gewöhnlich nur einige Sekunden beträgt, dann ist es klar, dass dieses nur dann einen merklichen Einfluss auf die Zahlenstreuung haben kann, wenn die Kokken mit ziemlich grosser Schnelligkeit ihrer Raumgitterlage zustreben würden. Man könnte dann erwarten, dass diese Erscheinung sich mikroskopisch beobachten liesse.

Um diesen Umstand untersuchen zu können, wurden einige Beobachtungen mit Mikroskop an Kokken in dichten Suspensionen in Bouillon und in Pferdeserum vorgenommen. Bei leichten Strömungen im Gesichtsfeld war jedoch keineswegs eine Beeinflussung der gegenseitigen Bewegung der Kokken zu konstatieren, obwohl hier Abstände in Betracht kamen, die viel kleiner waren als diejenigen, um die es sich in den Versuchssuspensionen handelte. Auch diesen Einfluss werden wir also vernachlässigen können.

Drittens werden wir berücksichtigen, dass der gestellten Forderung, dass p und q während der Probenentnahme der ganzen Serie konstant bleiben, nicht Genüge geleistet ist, und dass p so klein ist, dass annähernd $q = 1$ wird.

Gewöhnlich entspricht eine bakteriologische Probenentnahme dieser Voraussetzung ziemlich genau, aber bei restlosem Verbrauch der Suspension würde dies hingegen durchaus nicht so sein. Auch bei meinen eigenen Serien dürften p und q sehr inkonstant gewesen sein, da ich oft die vorläufigen Proben auf der Glasplatte durcheinander mischte, bevor ich daraus die endgültigen Proben entnahm. Obgleich dies für die Arbeitsweise von Frl. Ras nicht zutrifft, werden wir doch den extremen Fall, in dem eine Suspension restlos in vier Teile geteilt wird, näher betrachten, wobei $N = 400$, und $\widetilde{n} = 100$ ist.

Bei unendlicher Wiederholung der ganzen Probenentnahme würde für die

ersten Proben gelten: $\widetilde{u_1^2} = p_1 \cdot q_1 \cdot N = q_1 \cdot \widetilde{n}$ und $q_1 = 3/4$

zweiten " : $\widetilde{u_2^2} = p_2 \cdot q_2 \cdot N = q_2 \cdot \widetilde{n}$ " $q_2 = 2/3$

dritten und vierten " : $\widetilde{u_3^2} = p_3 \cdot q_3 \cdot N = q_3 \cdot \widetilde{n}$ " $q_3 = 1/2$

Bei Mittelung dieser Werte von $\widetilde{u^2}$ der ersten, zweiten, dritten und vierten Proben erhalten wir

$$\widetilde{u^2} = \frac{q_1 + q_2 + q_3 + q_4}{4} \cdot \widetilde{n} = \tag{17}$$

$$\frac{2{\cdot}416}{4} \cdot 100 = 60{\cdot}42$$

Weiter ist

$$\widetilde{x^2} = \frac{k-1}{k} \cdot \widetilde{u^2} = 3/4 \cdot 60.42 = 45.30 \text{ anstatt } 75 \text{ nach der Formel (12).}$$

Es ist ersichtlich, dass das korrigierte $\widetilde{x^2}$ dem $\overline{n}$ wieder proportional ist. Dieses $\widetilde{x^2}$ ist nebst dem nichtkorrigierten $\widetilde{x^2}$ graphisch in Abbildung 9 dargestellt wo zugleich gezeigt wird, wie meine eigenen Vierergruppen sich zu beiden mathematischen Erwartungen verhalten. Da der betrachtete extreme Fall den tatsächlichen Verhältnissen jedoch nicht ganz entspricht, liegt die wahre mathematische Erwartung irgendwo innerhalb der schattierten Zone. In der nächsten Abbildung ist dasselbe für meine Serien von 16 wiedergegeben.

In Bezug auf diese korrigierte mathematische Erwartung ist das mittlere x^2 in den Protokollen von Frl. Ras noch zu klein.

Wir werden nun auch das $\widetilde{S}_n$ auf dieselbe Weise korrigieren, indem wir in (14):

$$S_{(n)} = \sqrt{\widetilde{S}^2_{(n)}} \cdot \sqrt{\frac{2}{k-1}} \cdot \frac{T(\frac{1}{2}k)}{T(\frac{1}{2}k-\frac{1}{2})}$$

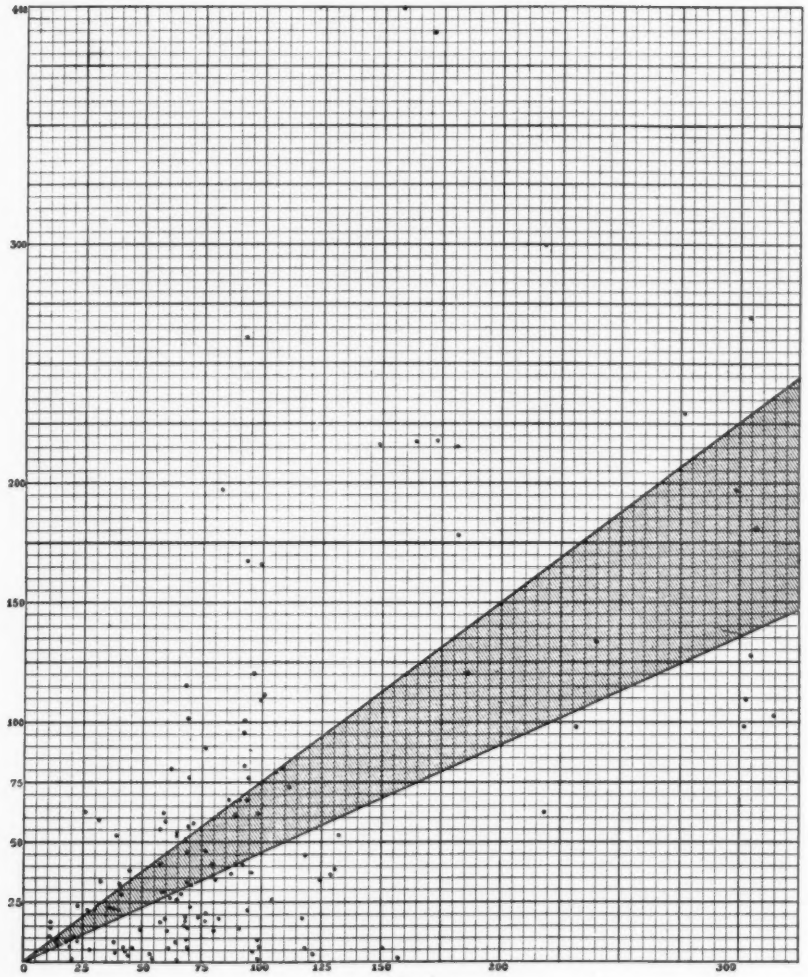

Abb. 9

(weil doch $\widetilde{S^2} = \widetilde{u^2}$) für $\widetilde{S^2_{(n)}}$ das korrigierte $\widetilde{u^2}$ substituieren. Für $k = 4$ und $\widetilde{n} = 100$ ergibt dies $\widetilde{S_{(n)}} = 4 \cdot 77$. Das auf diese Weise gleichfalls korrigierte $\widetilde{S_{(n)}}$ wird nebst seiner ursprünglichen Form in Abbildung 11 graphisch dargestellt. Es zeigt sich, dass das neue $\widetilde{S_{(n)}}$ bei $\widetilde{n} = 91.1$ 5% von $\widetilde{n}$ beträgt.

Erwägen wir, dass wir $\widetilde{n} = 80$ bis 100 als das üblichste Intervall betrachteten, so ergibt sich von neuem, dass die Daten der Ras' schen

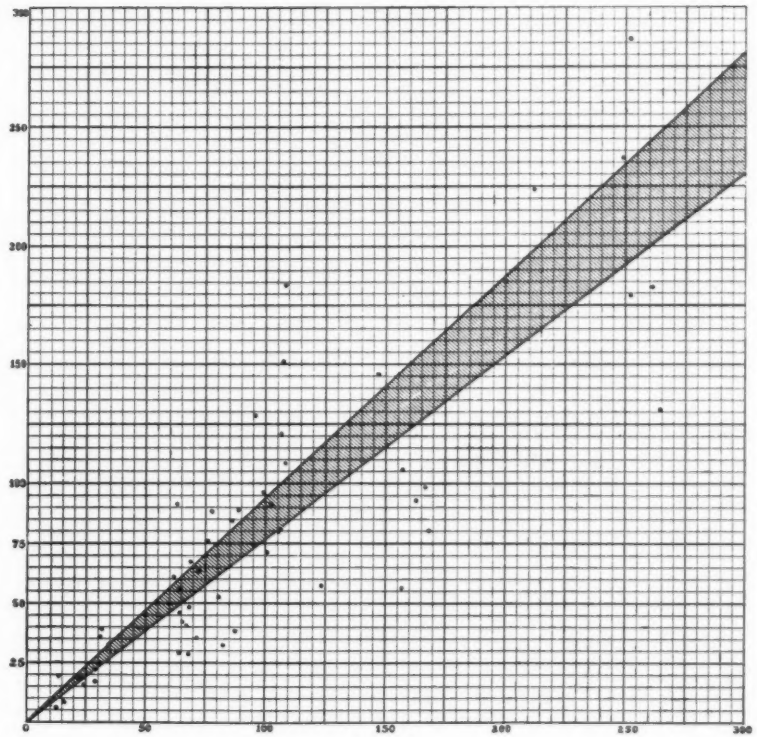

Abb. 10

Standard-Abweichung hiermit nicht im Einklang stehen. Zu diesem Befunde gelangen wir, trotzdem im betrachteten Fall die Korrektur, wie schon gesagt, grösseren Einfluss hat als in den diesbezüglichen Versuchen, sodass die wahre mathematische Erwartung auch hier wieder irgendwo in der schattierten Zone liegt.

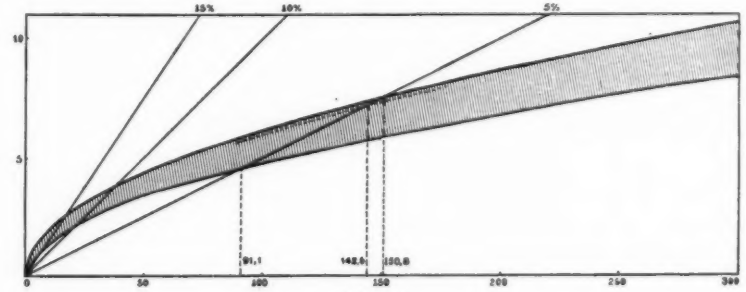

Abb. 11

Nach Vorschrift von Prof. Wolff sollten in dem Suspensionsbehälter nach der letzten Probenentnahme noch einige cm^3 der Suspension zurückbleiben, was noch ziemlich viel im Verhältnis zu dem Volumen der endgültigen Proben (30-50 mm^3) ist. Da Frl. Ras nun die vorläufigen Proben auf der Glasplatte *nicht* wieder mit einander mischte, lässt sich ihre wahre mathematische Erwartung wie folgt genauer abschätzen.

Wir nehmen den für Frl. Ras noch immerhin günstigen Fall an, dass

$$V = 1000\ mm^3$$
$$v = 50\ mm^3$$
$$\text{also } p_1 = \frac{v}{V} = \frac{1}{20}\ ;\ q_1 = \frac{19}{20}$$
$$q_2 = \frac{18}{19}$$
$$q_3 = \frac{17}{18}$$
$$q_4 = \frac{16}{17}$$

Setzen wir weiter $\widetilde{n} = 100$ voraus, so ergibt sich nach (17)

$$\widetilde{u^2} = \frac{\frac{19}{20} + \frac{18}{19} + \frac{17}{18} + \frac{16}{17}}{4} \cdot 100 = 94.58$$

$$\widetilde{x^2} = \frac{k-1}{k} \cdot \widetilde{u^2} = \tfrac{3}{4} \cdot 94{\cdot}58 = 70{\cdot}93$$

Weiter ist

$$\widetilde{S}_{(n)} = 0.614 \cdot \sqrt{\widetilde{u^2}} = 0.614 \cdot \sqrt{94.58} = 5.97.$$

Der unkorrigierte Wert von $\widetilde{S}_{(n)}$ ist $0.614 \cdot \sqrt{100} = 6.14$ und der extrem korrigierte war 4.77. Die reduzierte Korrektur von $\widetilde{S}_{(n)}$, die für die Arbeit von Frl. Ras zutrifft, beträgt also nur 12% der extremen. Rechnerisch lässt sich schliesslich ermitteln, das $S_{(n)}$ nun erst erst bei $\widetilde{n} = 142.6$ 5% von $\widetilde{n}$ beträgt.

Viertens könnten beim Füllen der „slide-cells" zwei Kokken so nahe aneinander geraten, dass sie am nächsten Tage zu einer gemeinsamen Kolonie ausgewachsen sind; man würde sie dann als eins zählen. Man würde also eine zu kleine Keimzahl finden, und der Fehlbetrag würde, wie leicht klar wird, bei den dicht besetzten Abteilungen bedeutender sein als bei den spärlicher besetzten derselben Serie. Wir dürfen erwarten, dass die Streuung daher etwas herabgesetzt wird.

Um den Einfluss zu studieren den diese Doppelkolonien auf die Frequenzverteilung haben, betrachten wir eine Gruppe von Proben mit gleichem n, die aber von Suspensionen mit verschiedenem $\tilde{n}$ stammen.

Wir werden nun bestimmen, welche mittlere Keimzahl n wir beim Auszählen unendlich vieler Proben dieser Gruppe erhalten würden. Wir wollen also eine mathematische Erwartung ermitteln, und dazu werden wir die Frequenzen aller Ereignisse wieder ihren Wahrscheinlichkeiten proportional setzen.

Falls irgend zwei Kokken nahe bei einander liegen, wird unser Fazit beim Auszählen der Kolonien einen Fehlbetrag enthalten. Da es rechnerisch gleichgültig ist, welche Individuen zusammen ein Paar bilden, ist es klar, dass man denselben Fehlbetrag auf verschiedene Weise bekommen kann.

Die mathematische Erwartung des gesamten Fehlbetrages stellt sich als Summe aller mathematischen Erwartungen der Fehlbeträge dar, die infolge der verschiedenen möglichen Paare auftreten können. Die mathematische Erwartung des Fehlbetrages ist für jedes mögliche Paar gleich dem Produkte des Defizits und der Wahrscheinlichkeit desselben. Da bei jedem Paar das Defizit eins beträgt, summieren wir einfach die Wahrscheinlichkeiten aller möglichen Paare in Proben mit n Kokken.

Die Anzahl der möglichen Paare beträgt nach Formel (4)

$$C_n^2 = \frac{n!}{2!(n-2)!} = \frac{n(n-1)}{2} = \tfrac{1}{2} \cdot n \cdot (n-1)$$

Befindet sich ein Kokkus auf einer gewissen Stelle einer „slide-cell"-Abteilung, so ist die Wahrscheinlichkeit, dass ein bestimmter anderer Kokkus auf eine Stelle geraten wird, die weniger als r von der ersten entfernt ist, gleich

$$p = \frac{o}{O}$$

Hier ist o die Oberfläche des Kreises um den ersten Kokkus, mit Radius r; r ist die Minimalentfernung, die zwei Kokken haben müssen, damit am nächsten Tage ihre Kolonien noch getrennt sichtbar sind. Nach meinen eigenen Beobachtungen ist $r = 0{,}3$ mm.

Weiter ist O die Oberfläche einer „slide-cell"-Abteilung, die in unserem Fall $15 \cdot 25 = 375$ mm^2 beträgt.

So hat man

$$p = \frac{\pi r^2}{O}$$

Die mathematische Erwartung des gesamten Fehlbetrages ist also gleich:

$$C_n^2 \cdot p = \tfrac{1}{2} \cdot n \cdot (n—1) \cdot \frac{\pi r^2}{O} = \qquad (18)$$

$$0{\cdot}0003769 \cdot n \cdot (n—1)$$

Für $n = 90$ ergibt sich

$$0.0003769{\cdot}90{\cdot}89 = 3.02$$

Beim Auszählen der Kolonien nähert sich das scheinbare $\overline{n}$ dem Werte 86.98.

Für $n = 110$ ergibt sich

$$0.0003769{\cdot}110{\cdot}109 = 4.52$$

Beim Auszählen der Kolonien nähert sich das scheinbare $\overline{n}$ dem Werte 105.48.

Das Intervall $110—90 = 20$ wird beim Zählen der Kolonien also zusammengedrückt bis $105.48—86.98 = 18.50$, sodass es sich nun auf $\frac{18.5}{20.0}$ oder 0.925 seines ursprünglichen Wertes stellt.

Da nun $\widetilde{S}$ wie n beide gleicher Dimension sind, dürfen wir annehmen, dass auch $\widetilde{S}$ infolge der Bildung von Doppelkolonien (wenigstens ungefähr) 0.925 seines ursprünglichen Wertes beträgt, wenn $\widetilde{n}$ unweit 100 ist. Wenden wir dies auf das $\widetilde{S}$ mit reduzierter Korrektur an (infolge Inkonstanz von p und q) so erhalten wir

$$\widetilde{S}_{(n)} = 0.925{\cdot}5{\cdot}97 = 5.52,$$

was noch immer grösser ist als das extrem korrigierte $S_{(n)} = 4.77$.

Es ist also klar, dass das Zusammenwirken aller bis jetzt berücksichtigten Faktoren nicht im Stande ist, die geringe Zahlenstreuung der Ras' schen Protokolle zu erklären.

Fünftens könnten die Kokken anfangs ihre Vitalität gegenseitig beeinflussen; ein schwacher Kokkus, dessen Schicksal noch ungewiss ist, könnte durch die Nachbarschaft eines anderen Kokkus in seiner Entwicklung angeregt oder niedergehalten werden.

Im ersten Fall würde dieser Umstand eine Tendenz zur Gruppenbildung, und damit zu einer grösseren Zahlenstreuung zur Folge haben.

Im letzten Fall gäbe es eine Neigung zu einer gitterähnlichen Lage der Kolonien, was die Streuung herabsetzen würde. Nur dieser letztere Fall könnte in Bezug auf die vorliegenden Ausführungen wichtig sein.

Da die Physiologie der Kokken hier eine Rolle spielt, is es ohne weiteres ersichtlich, dass diese Angelegenheit sich nicht a priori erörtern lässt. Ich kann jedoch meine eigene Empirie beibringen; ich arbeitete nämlich mit denselben Kokkenstämmen und mit denselben Kulturmedien. Daher hätte sich dieser Einfluss in meinen Daten gleich deutlich zeigen müssen. Zwar waren meine Kulturmedien namentlich im Anfang den Utrechtern in Einzelheiten nicht ganz gleich, aber dazu lässt sich bemerken, dass ich auch mit den Utrechter Ingredienzien immer denselben Erfolg hatte. Übrigens wurden in Utrecht wiederholt erhebliche Änderungen in der Zusammensetzung der Nährböden eingeführt, was angeblich ganz nebensächlich sein sollte (Siehe meine Originalarbeit S.11).

Sechstens könnte die Zahlenstreuung klein werden, indem man diejenigen der gefundenen Werte streicht, die mehr vom Durchschnitt abweichen, als man als Laie auf dem Gebiete der Wahrscheinlichkeitsrechnung erwartet. Man könnte nämlich leicht annehmen, dass hier irgend eine Unregelmässigkeit vorgekommen ist, und so ist es vom Laienstandpunkt gerechtfertigt, solch einen Wert ausserhalb der Berechnung zu lassen, da eine gleiche Verwertung aller Daten auf der Annahme beruht, dass wir allen gefundenen Werten gleiches Vertrauen beimessen dürfen.

Gewiss sind aus diesem Grunde auch in meinen Daten einige extreme Werte gestrichen worden. Ich halte es jedoch nicht für möglich, dass sich die Eigentümlichkeiten der Utrechter Zahlenstreuung in dieser Weise erklären lassen, da die Assistenten verhältnismässig viele unverletzte Serien lieferten.

Siebentens könnte Autosuggestion eine gewisse Rolle spielen. Das Auszählen der "slide-cells" ist nämlich nicht vollkommen genau, da sich die Kolonien durchaus nicht immer als scharf von einander abgegrenzte Einheiten zeigen. In solchen Fällen muss dann bei einigen Koloniengruppen die Schätzung zu Hilfe genommen werden, sodass es sehr wohl passieren kann, dass zwei Zähler bona fide sich über die richtige Zahl nicht einig werden können.

Nun sind die normalen Abweichungen viel grösser, als auch ich damals als Laie auf dem Gebiete der Wahrscheinlichkeitsrechnung erwartete, und aus Erfahrung weiss ich, wie leicht man sich dabei ertappen kann, dass man der Tendenz, die Zahlenstreuung dadurch kleiner zu taxieren, erliegt. Diese Beeinflussung wird am grössten bei kleinen Serien sein da sich diese am besten übersehen lassen. Man vergleiche hierzu die Figuren 5 und 6; vorübergehend wurde schon erwähnt, dass hier bei den Vierergruppen verhältnismässig mehr Punkte unter die Linie fallen. Doch bin ich der Meinung, dass bei Experimentatoren bona fide dieser Zählfehler nur einige Prozente

betragen wird, sodass man hierdurch nicht solche Streuungen bekommen kann, wie diejenigen der Utrechter Protokolle.

Wir sehen also, dass alle betrachteten Faktoren nicht imstande sind, die Eigentümlichkeiten der Zahlenserien des Wolffschen Laboratoriums zu erklären. Lange habe ich mich bemüht, immer noch eine Erklärung zu finden; man sollte es mir nicht übelnehmen, dass ich nur noch eine einzige Erklärung für möglich halte, nämlich

Achtens: dass die Personen, die mit der Ausführung der Experimente betraut waren, die wahre Bedeutung ihrer Arbeit nicht ermessen haben. Im Verlauf der vorliegenden „Strahlungs"-forschung habe ich mich zu meinem Bedauern gezwungen gesehen, auch diese weniger angenehme Erklärungsmöglichkeit in Erwägung zu ziehen.

Weiland Prof. L. K. Wolff hielt eine winzige Streuung für das massgebende Kriterium einer guten Versuchstechnik. Da er die Beobachtungen nicht selber vornahm, wurde es ihm nicht zur rechten Zeit klar, dass die Streuung sogar bei tadellos genauem Arbeiten viel grösser ist, als er seiner Meinung nach für möglich hielt. Seine Laboranten, denen viel daran liegen musste, ihren Prinzipal zu befriedigen, haben sich vermutlich nicht gescheut, Zahlen zu notieren, die die erwünschte Streuung aufwiesen. Es liesse sich dies zwar verstehen, aber von wissenschaftlichem Standpunkte keineswegs entschuldigen. Es ist selbstverständlich, dass man Daten, die unter solchen bedauerlichen Umständen erzielt worden sind, jegliche Beweiskraft absprechen muss.

In dieser Weise denke ich mir den Hergang; sollte ich mich irren, so möge man das in einer Veröffentlichung klar machen. Beim Erscheinen meiner Doktorarbeit zeigten sich Prof. Wolff wie Prof. Gurwitsch in ihrer Privatkorrespondenz besonders empört über die Kritik, die ich an ihren Ansichten ausübte, aber es unterblieb bis jetzt jegliche Publikation irgend einer Erwiderung auf wissenschaftlicher Grundlage.

Vom Vertrauen, das ich anfangs zu der Zuverlässigkeit der bisherigen Forschungen der mitogenetischen Strahlung hatte, war mir beim Abschliessen meiner Arbeit immer noch ein gewisser Rest übrig geblieben. Aber auch dieser Rest verschwand, als Prof. Gurwitsch mir bald nach dem Erscheinen meiner Publikation brieflich mitteilte[8], dass man in seinem Laboratorium mit der Hefesprossmethode seit Jahren zu Serien kommt, die meistens sogar noch glatter sind als die Utrechter. Es wurden drei solche Serien angeführt, nämlich

54, 55, 53, 53, 50.
52, 56, 54, 54, 58, 56, 55.
56, 51, 55, 59, 55, 57, 63.

[8] Leningrad, den 25sten Dezember 1935.

Überlegen wir, in wieweit unsere Betrachtungen sich auf die Serien der Hefesprossmethode anwenden lassen.

Die Anzahl kleiner Knospen (n) wird auf je (N) =1000 Zellen abgezählt. Teilen wir die Gesamtzahl der Knospen durch die Gesamtzahl der Zellen im Präparat, so erhält man den Knospengehalt, p.

Nun ist $\widetilde{n} = p.N$

Die Wahrscheinlichkeit, dass eine bestimmte Zelle eine Knospe trägt, is p. Die Wahrscheinlichkeit, dass zwei bestimmte Zellen eine Knospe haben, beträgt p^2. Die Wahrscheinlichkeit, dass n bestimmte Zellen eine Knospe tragen ist p^n. Dass *nur* diese n Zellen eine Knospe tragen $p^n \cdot q^{N-n}$; dass nur *irgend n* Zellen eine Knospe tragen

$$C_N^n \cdot p^n q^{N-n} = \frac{N!}{n!(N-n)!} p^n \cdot q^{N-n}$$

und so gelten weiter alle Überlegungen auch für die Zahlen der Hefesprossmethode.

Die Korrekturen eins bis fünf treffen hier sogar nicht zu, denn die Zellen werden beim Anfertigen des Präparates gemischt, aber bleiben beim Zählen an fester Stelle fixiert, und warten ruhig ab, bis sie an die Reihe kommen; p und q bleiben also konstant. Da die Zellen nicht in der Lage gezählt werden, in der sie ihre Entwicklung auf dem Agarboden anfingen, kommt auch die fünfte Korrektur nicht in Betracht. Auch hier also wieder eine zu geringe Zahlenstreuung!

Wenn sich so etwas im Hauptquartier der mitogenetischen Strahlungsforschung vorfindet, überschleicht einen ein Gefühl des Misstrauens in alle „Beweise" der mitogenetischen Strahlung, einschliesslich solcher, die man angeblich mit der Zwiebelwurzelmethode erzielt haben sollte. Die Streuung der Mitosen lässt sich zwar nicht a priori erörtern, aber wo man schon mit Kokken oder Hefen so glatt arbeitet, kann man von Zwiebelwurzeln oder sonstigen „Detektoren" auch alles erwarten.

Am Ende möchte ich mich Hollaender (5) anschliessen, der auf Grund weitgehender Experimente zum Schlusse kam, dass die „Beweise" der Existenz der mitogenetischen Strahlung nicht auf wissenschaftlicher Grundlage beruhen. Mehr lässt sich zur Zeit nicht davon sagen, denn das Nicht-Existieren eines Phänomenes lässt sich nun einmal nicht experimentell nachweisen.

ZUSAMMENFASSUNG

Vorliegende Arbeit betrifft eine Nachprüfung der sogenannten "slide-cell"-Methode von Wolff und Ras zum Nachweis der mitogenetischen Strahlung. Bei dieser Methode versucht man in einer Bakteriensuspension eine Keimzahlvermehrung nachzuweisen, die infolge einer Bestrahlung auftreten sollte. Im *ersten Abschnitt* wird erörtert, dass sich trotz peinlichster Nachahmung der angegebenen Versuchsbedingungen, nur zweierlei Ergebnisse feststellen liessen: Erstens gelang es nicht, eine regelmässig reproduzierbare Vermehrung der Bakterien hervorzurufen. Zweitens war die Streuung, die man beim Auszählen von Probenserien einer Bakteriensuspension erhält, viel grösser, als sie nach Angaben von Wolff und Ras sein sollte. Dieser Umstand veranlasste mich, meine Versuchsfehler eingehend zu untersuchen. Einerseits wurden die Fehlerquellen beachtet, die die Konzentration der Suspensionsproben beeinflussen könnten, nämlich Verdunstung und ungenügende Mischung. Andererseits überwachte ich meine Fehler beim Abmessen der Probenvolumen. Sämtliche Fehlerquellen waren aber nicht im Stande, meine tatsächlich erhaltenen Zahlenstreuungen zu erklären. Daher widmete ich mich dem mathematischen Studium der Streuung. Es wird im *zweiten Abschnitt* gezeigt, dass man bei gewissen Voraussetzungen a priori quantitative Aussagen über die Streuung, die man bei idealer fehlerlosen Arbeit erwarten muss, machen kann. Das Zahlenmaterial wird nach zwei verschiedenen Kriterien geprüft, und es stellt sich dabei heraus, dass meine Daten mit den mathematischen Erwartungen ziemlich gut übereinstimmen, die Streuung in den Ras' schen Protokollen dagegen eine viel geringere ist. Im *dritten Abschnitt* wird untersucht, ob diese glatten Zahlenserien sich vielleicht dadurch erklären lassen, dass die gemachten Voraussetzungen den wahren Versuchsverhältnissen nicht genügend entsprechen. Es werden daher einige Korrekturen eingeführt, welche die Ras' sche Streuung aber noch immer nicht erklären können. Daraus meine ich schliessen zu müssen, dass man den Daten von Frl. Ras jegliche Beweiskraft absprechen muss. Dasselbe gilt für das übliche Zahlenmaterial aus dem Laboratorium von Prof. Gurwitsch.

Abgeschlossen im März 1939.
Batavia.

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THE GROWTH ACTIVATING EFFECT OF EXTRACTS OF ADULT TISSUE ON FIBROBLAST COLONIES IN VITRO

II. EXPERIMENTS WITH EXTRACTS OF BRAIN, BONE-MARROW, LIVER, LUNG, SMOOTH MUSCLE, PANCREAS, KIDNEY, OVARY, OVIDUCT, SPLEEN AND TESTIS*

By

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(*Received for publication November 29, 1939*)

In part I of this investigation (Hoffman and Doljanski, 1939) we showed that aqueous extracts of stable, mitotically inactive, tissues stimulate growth of fibroblast cultures in vitro. The growth activating effect of heart extracts of adult chickens was determined quantitatively. As a continuation of these experiments we tested the growth stimulating properties of other organs and tissues.

MATERIAL AND PROCEDURE

The method of preparing the extracts as well as the culture methods remain practically the same as those described in part I, except that instead of the Latapie apparatus scissors were used to cut the tissues very finely. The tissue pulp was extracted with 4 times its volume Tyrode and the extracts were tested in the original concentration. The cell growth stimulating effect of extracts was determined by measuring the growth intensity of fibroblast colonies in vitro. Experiments were always carried out with sister halves of cultures of which one half was placed in protective medium (Plasma-Tyrode), the other half was cultivated in protective medium plus the tissue extract being investigated. The activity of the extracts of these various organs and tissues was also compared with that of heart extract.

EXPERIMENTS AND RESULTS

In the experiments here described extracts of the following tissues and organs were tested for their cell-growth promoting activity: brain, bone-marrow, liver, lung, smooth muscle (gizzard), spleen, kidney, ovary, oviduct, pancreas and testis. *All these extracts were found to markedly stimulate the growth of fibroblasts in vitro.*

*A preliminary note on this subject has been published (1939).

The curves in Figure 1 illustrate the action of each of the extracts tested. These curves were selected from the experimental material with the view of showing the optimum cell growth stimulation in each case.

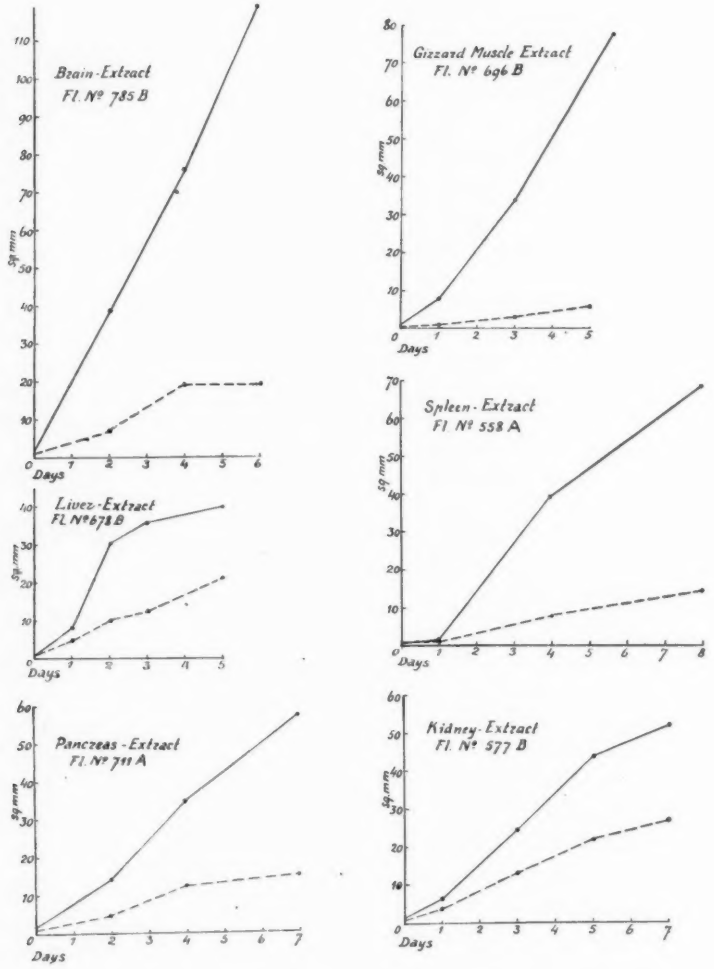

Fig. 1a. Growth rate of sister halves of fibroblast cultures growing in tissue extract media and in protective medium.

——— tissue extract media

- - - - - - - - protective medium

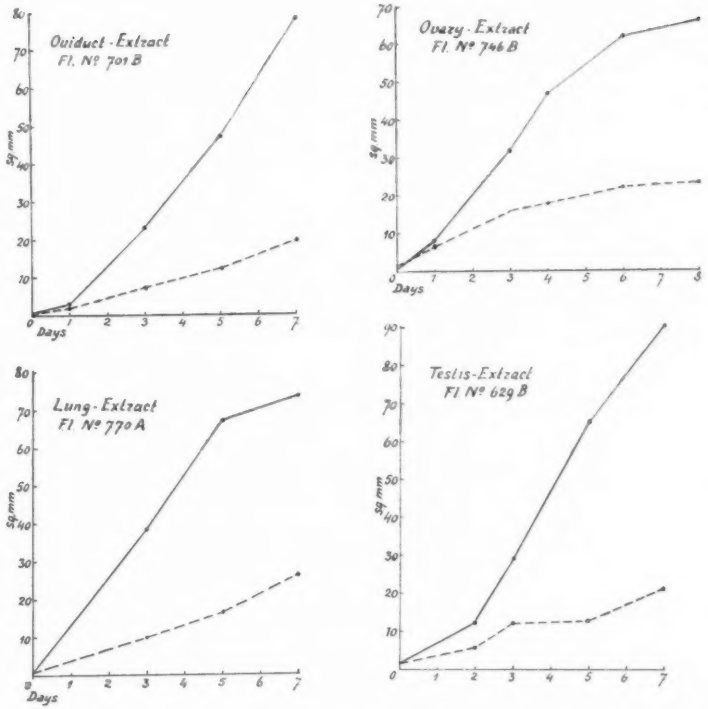

Fig. 1b. Growth rate of sister halves of fibroblast cultures growing in tissue extract media and in protective medium.

——— tissue extract media
- - - - - - - - protective medium

Table I gives a picture of the comparative growth of cultures growing in medium with the addition of different extracts as compared with their sister halves growing in heart extract. It is hardly possible to give a standard value of the degree of stimulation of each of the tissue extracts, for extracts of the same tissue may vary as to degree of activity and the cell cultures also show considerable individual variation in their reaction. However, by comparing the activity of each extract with that of heart extract it is possible to form some quantitative idea of their relative strength.

Of all the extracts tested brain extract has the greatest capacity for stimulating the growth of cell cultures, almost twice that of heart extract. The growth stimulating effect of extracts of gizzard muscle is very similar to that of heart extract, although in most cases it is more

TABLE I

THE SIZE OF CULTURES IN mm^2 ON THE 6TH DAY

No. of Culture	Brain Extract	Cardiac Muscle Extract	No. of Culture	Gizzard Muscle Extract	Cardiac Muscle Extract
783a	76	52	697a	94	88
783b	77	65	697b	118	60
784a	70	25	827a	97	56
784b	65	55	827b	48	36
786a	97	51	828a	59	25
786b	100	33	828b	25	21
856a	102	40	829a	59	65
856b	52	43	829b	49	35
857a	85	59	830a	42	60
857b	72	64	830b	71	71
	mean 80	49		mean 66	52

No. of Culture	Ovary Extract	Cardiac Mus. Ex.	No. of Culture	Lung Extract	Cardiac Mus. Ex.
630a	49	50	732a	29	57
630b	42	54	732b	31	70
738b	27	64	733a	32	49
739b	70	41	733b	23	37
747a	51	39	744a	47	86
747b	66	69	744b	30	40
861a	54	63	760a	41	54
861b	62	46	760b	44	66
862a	59	33	769a	35	43
862b	50	37	769b	27	69
863a	61	67	792a	57	55
863b	43	59	792b	49	48
	mean 53	52		mean 37	55

No. of Culture	Pancreas Extract	Cardiac Mus. Ex.	No. of Culture	Kidney Extract	Cardiac Mus. Ex.
725a	34	35	592a	23	41
725b	52	36	626b	17	47
714a	38	50	639a	35	65
714b	30	53	666a	34	50
726a	33	50	666b	32	64
726b	42	52	667b	16	48
864a	26	34	694a	25	38
867a	18	39	695a	30	69
869a	45	46	719a	24	51
869b	41	60	719b	30	62
870a	25	63	742b	16	38
870b	20	72	743a	24	42
	mean 33	49		mean 25	51

TABLE I (Cont.)

No. of Culture	Liver Extract	Cardiac Muscle Extract	No. of Culture	Bone-marrow Extract	Cardiac Muscle Extract
646a	8	41	657b	16	43
646b	20	41	659a	33	30
647a	13	27	815a	20	60
647b	19	25	815b	30	49
654a	8	32	823a	24	26
654b	19	38	823b	27	88
681a	14	60	824a	33	70
681b	21	70	824b	31	75
781a	11	81	825a	16	29
781b	12	97	825b	54	55
795a	38	64	826a	28	70
795b	21	54	826b	23	32
	mean 17	52		mean 28	52

No. of Culture	Testis Extract	Cardiac Muscle Extract	No. of Culture	Spleen Extract	Cardiac Muscle Extract
599a	23	24	598a	32	56
599b	26	31	598b	44	61
628a	22	30	717a	43	63
628b	23	44	717b	67	100
754a	17	58	723a	71	37
754b	38	95	723b	38	47
755a	71	71	748a	22	52
755b	36	76	749b	36	60
765a	45	75	763a	20	53
765b	45	67	763b	21	54
766a	39	62	774a	26	52
766b	38	80	774b	84	85
	mean 35	59		mean 42	60

active. Ovarian extract is just about the same as heart extract in its stimulating action.

Lung, spleen, pancreas and testis extracts stimulate the growth of cultures to a lesser degree than heart extract while extracts of bone-marrow, liver and kidney have considerably lower stimulating capacity even than these.

The structure and appearance of fibroblast colonies to which extract of ovary, smooth muscle, lung, spleen or testis has been added are similar to colonies growing in medium containing heart extract. They are regular in structure; the cells are only slightly granulated. Cultures stimulated with brain extract differ somewhat from these in

appearance. These colonies are especially uniform in appearance and transparent; the cell granules are hardly apparent. On the other hand cultures growing in media which contain pancreas, bone-marrow, kidney and liver extracts are quite dark and compact and the cells are full of granules. Fig. 2-11 give a general picture of the size and appearance of cultures in various tissue extracts compared in some cases

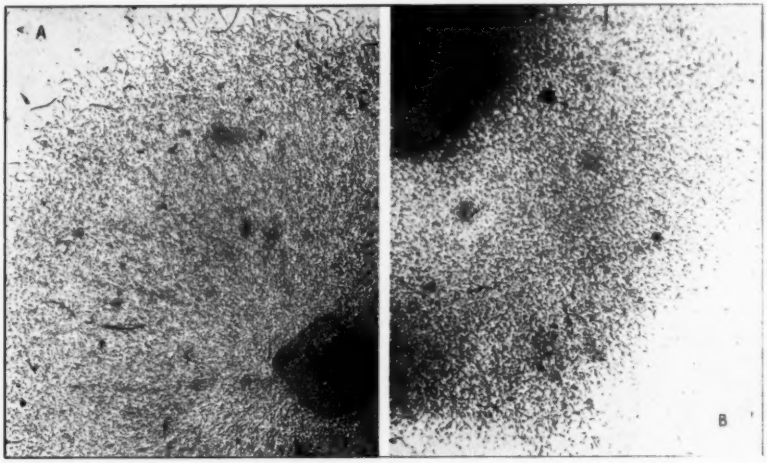

Fig. 2, No. 860. A—8 day old fibroblast culture in brain extract medium. B—sister half, same age, in heart extract medium, living, × 12.

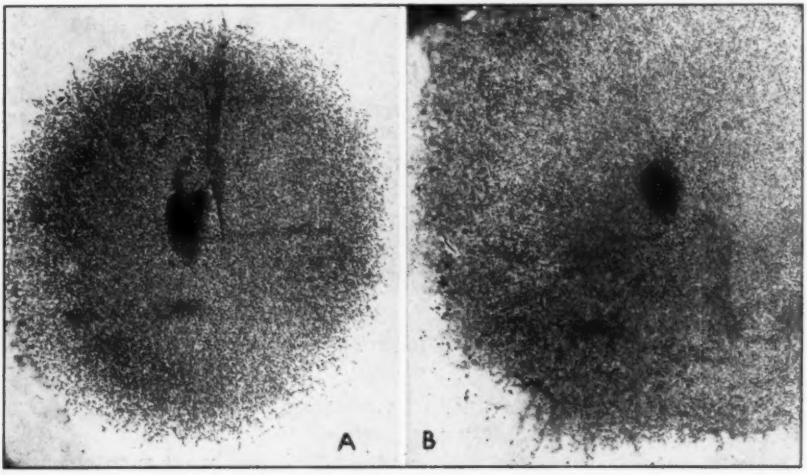

Fig. 3, No. 815. A—7 day old fibroblast cultures in bone marrow extract medium. B—sister half, same age, in heart extract medium, living, × 15.

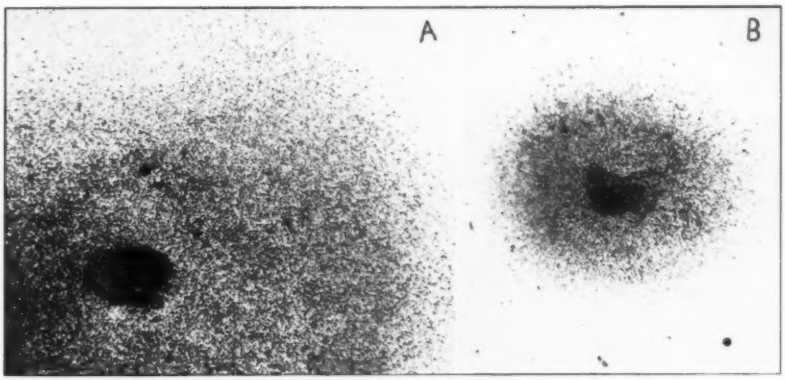

Fig. 4, No. 770. A—6 day old fibroblast culture in lung extract medium. B—sister half, same age, in protective medium, living, × 13.

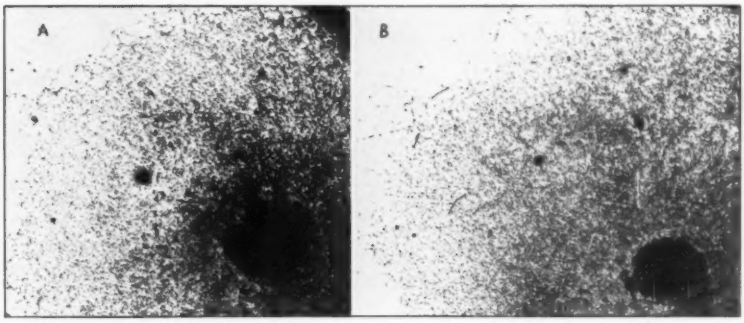

Fig. 5, No. 830. A—7 day old fibroblast culture in gizzard muscle extract medium. B—sister half, same age, in heart extract medium, living, × 12½.

with sister halves growing in heart extract medium and in other cases with those in protective medium.

The experiments show clearly that *the capacity to stimulate the growth of cultures in vitro is present in a variety of tissues and organs.* Though there is a diference between the intensity of action of the different extracts that we tested, they are all capable of stimulating cell growth.

In looking over the stimulation values of the extracts of various tissues in Table I, one notices that there is no relation between the ability of the different organs and tissues to stimulate the growth of cell colonies in vitro and the mitotic activity in these tissues. Brain which is par excellence an example of a mitotically inactive tissue (tissue with

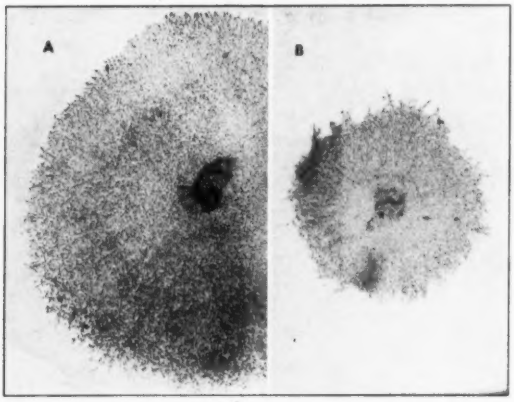

Fig. 6, No. 775. A— 6 day old fibroblast culture in spleen extract medium. B—sister half, same age, in protective medium, living, × 13.

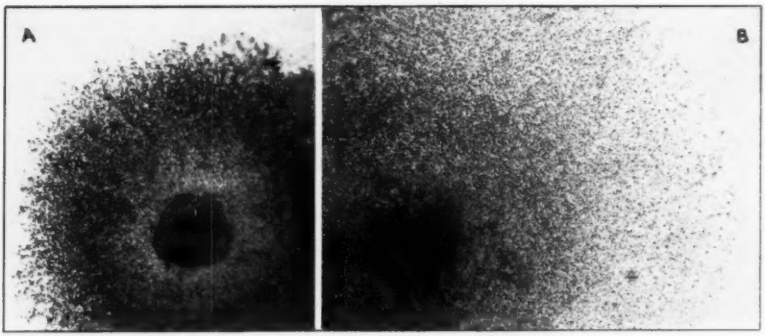

Fig. 7, No. 694. A—7 day old fibroblast culture in kidney extract medium. B—sister half, same age, in heart extract medium, living, × 12.

unchanging elements—according to the classification of Bizzozero-Levi) yields an extract with the greatest growth stimulating effects (Fig. 12). On the other hand the activity of extracts of mitotically active organs of tissues which are in a continued state of cell formation (tissues with labile elements according to Bizzozero-Levi) such as bone-marrow and testis is less than that of heart extract (Fig. 13).

In the light of these findings as well as those showing that heart extract has a greater stimulating action (in the same concentration) than embryonic extract on fibroblast cultures in vitro the conception that extracts of mitotically active tissue have a much greater growth

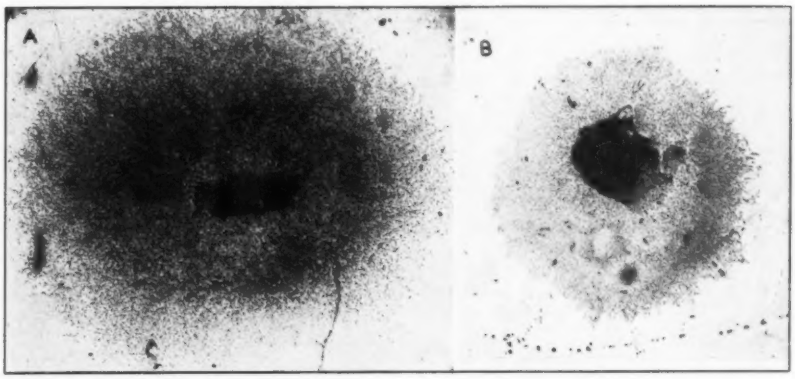

Fig. 8, No. 746. A—4 day old fibroblast culture in ovary extract medium. B—sister half, same age, in protective medium, living. × 11.

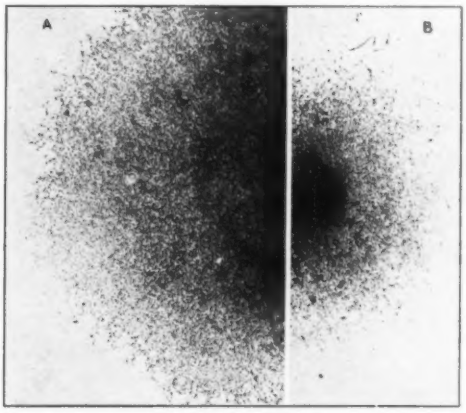

Fig. 9, No. 701. A—7 day old fibroblast culture in oviduct extract medium. B—sister half, same age, in protective medium, living. × 13.

activating effect than extracts of stable tissue is entirely without foundation. *Extracts of tissue of adult organism in physiological state of proliferation are not more active than extracts of stable tissues.*

It is not without interest to emphasize in this connection also that extracts of spleen, an organ containing a large quantity of white blood cells which according to Carrel (1922) are an important source of growth stimulating substances in the adult organism, have no exceptional growth stimulating powers. Actually the growth stimulating action

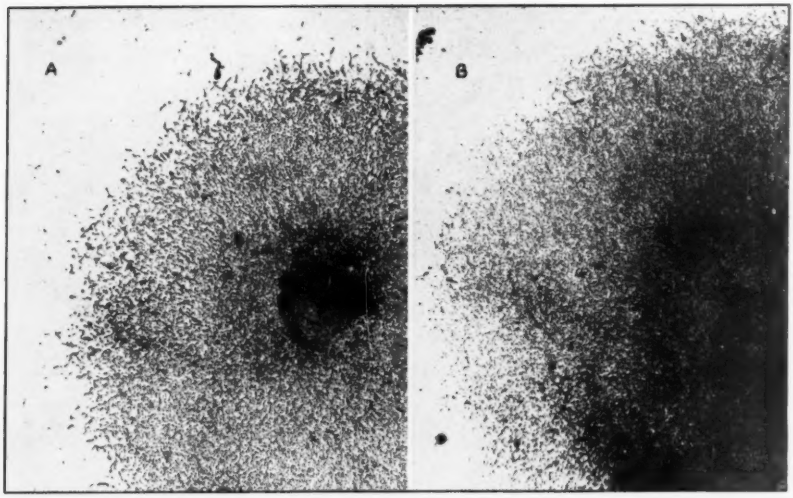

Fig. 10, No. 869. A—6 day old fibroblast culture in pancreas extract medium. B—sister half, same age, in heart extract medium, living. × 14.

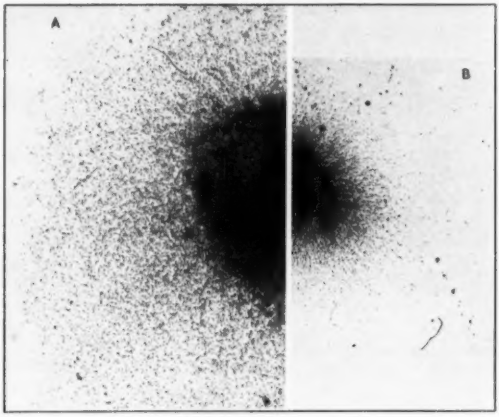

Fig. 11, No. 629. A—9 day old fibroblast culture in testis extract medium. B—sister half, same age, in protective medium, living. × 16.

of spleen extract is considerably less than that of heart extract. The curves in Fig. 14 show this relationship.

It is important to emphasize the fact that extracts of *all* organs and tissues we tested have the capacity to stimulate growth of cells *in vitro*. Liver extract is no exception to this rule. We mention this fact because

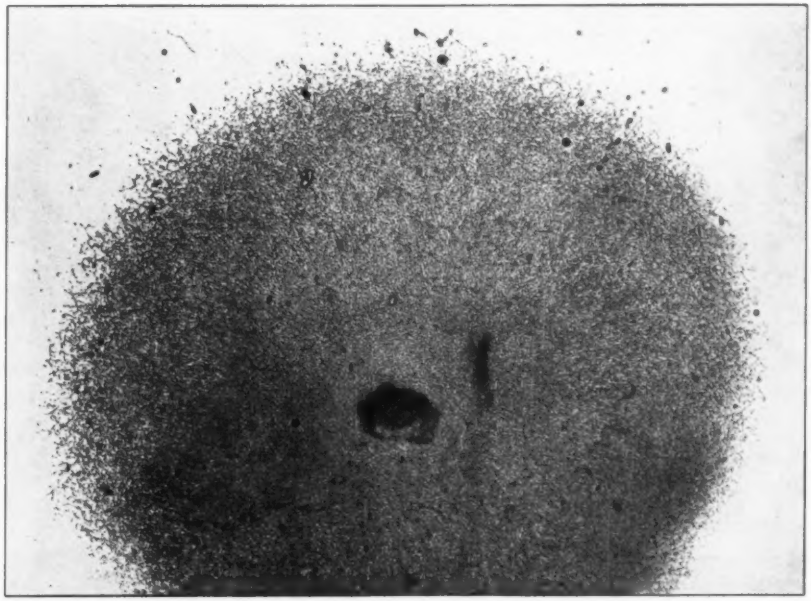

Fig. 12, No. 856. 8 day old fibroblast culture in brain extract medium, living. × 10.5.

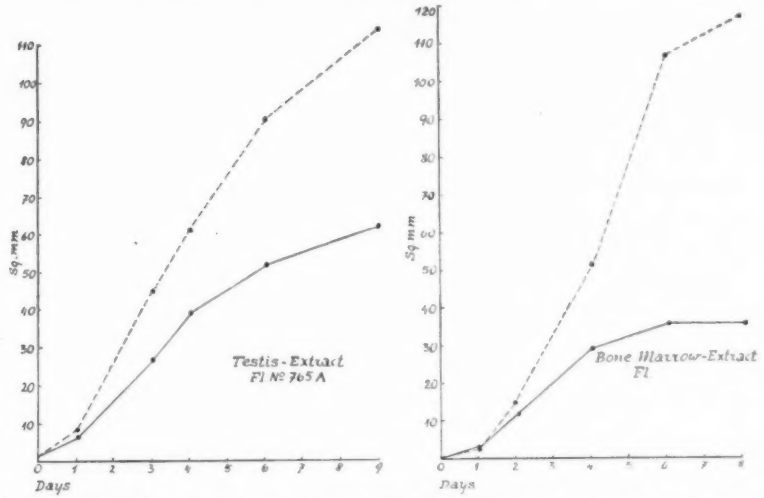

Fig. 13. Growth rate of sister halves of fibroblast cultures in testis and in bone-marrow extract media compared with sister halves in heart extract medium.

———— testis or bone-marrow extract medium

- - - - - - - - heart extract medium

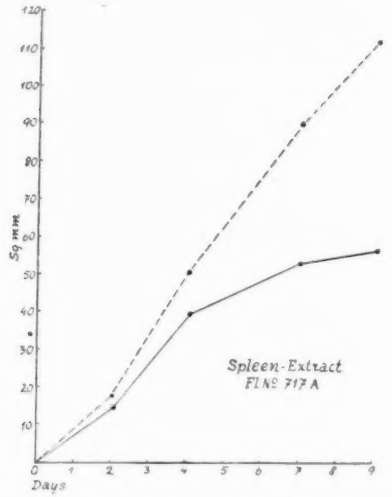

Fig. 14. Growth rate of sister half of fibroblast culture in spleen extract medium compared with sister half in heart extract medium.

——— spleen extract medium
- - - - - - - - heart extract medium

several papers have been published attributing growth inhibitory properties to aqueous extracts of liver.

Walton (1914) was the first to ascribe a growth inhibitory effect to liver extract. Lynch (1921) and Lewis (1925) because they obtained no growth in vitro of liver of 16 day old chick embryo suspected that liver tissue contained a growth inhibitor. Recently Brues, Jackson and Aub (1936) in a series of experiments compared the growth of fibroblasts in protective medium with that in medium containing liver extract. They showed that on the addition of liver extract diluted 1:2 the growth of cell colonies was inhibited 85%. With the addition of liver extract diluted 1:6 to 1:8 cell growth was inhibited from 30 to 50%. The authors also attempted to determine the chemical nature of the growth inhibitor in liver tissue.

As we have already shown, liver definitely stimulates growth but this effect is smaller than in the case of some other tissue extracts. The stimulating action is evident from the comparison of the growth curves, during the first few days, in media containing liver extract with their sister halves growing in protective medium (Table II). On the 4th day in most cases there is a marked flattening of the growth curve and on the 5th to 6th day a complete stoppage of growth. At this stage

TABLE II
THE SIZE OF CULTURES IN MM²

No. of Culture	On 3rd Day		On 5th Day	
	Protective Medium	Liver Extract Medium	Protective Medium	Liver Extract Medium
650a	5	9	10	19
650b	7	12	12	20
651a	4	8	7	20
651b	3	12	5	21
652a	3	9	9	19
662b	2	8	6	17
672a	4	17	6	28
678a	10	30	17	38
678b	12	18	16	26
731a	6	12	13	30
797b	8	13	9	16

fibroblast cultures growing in liver extract are markedly degenerated as compared to younger cultures in liver extract (Fig. 15, 16, 17).

We reserve for later consideration the problem of whether the subsequent slowing down and final checking of growth is due to some special inhibiting factor present in liver extract. It will be considered in connection with a general investigation of the problem of variation in the growth stimulating action of various organs and tissues. Two possibilities may be considered: either that the various tissues contain different quantities of growth promoting agents, or that, although the content of growth promoting agents is the same in all tissues, there are in addition a varying amount of specific growth inhibitors or specific factors injurious to cells.

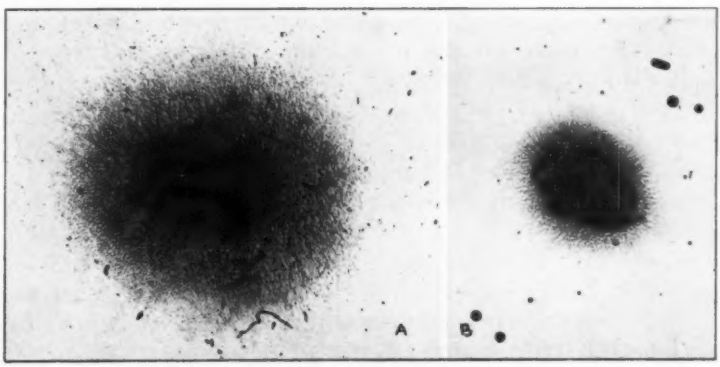

Fig. 15, No. 672. A—2 day old fibroblast culture in liver extract medium. B—sister half, same age, in protective medium, living. × 16.

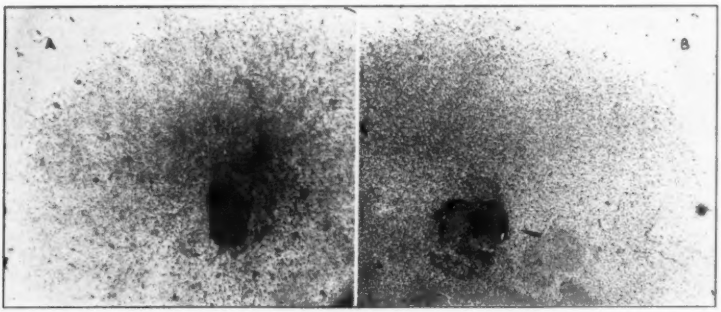

Fig. 16, No.245. A—4 day old fibroblast culture in liver extract medium. B—sister half, same age, in heart extract medium, living. × 18.

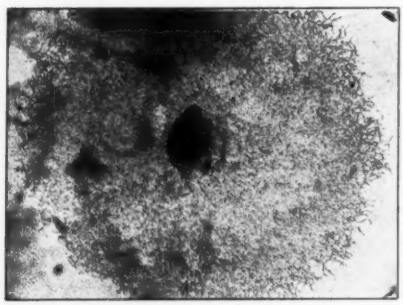

Fig. 17, No. 795. 6 day old fibroblast culture in liver extract medium, living. × 16.

SUMMARY

It was shown that extracts of brain, testis, ovary, lung, smooth muscle, pancreas, spleen, bone-marrow, kidney and liver of adult chicken have marked growth stimulating effect on cultures of fibroblasts in vitro.

The degree of activity of the various tissue extracts investigated differed. The greatest growth stimulating effect was obtained by the addition of brain extract. The other organ extracts may be listed as follows in the order of the degree of their activity: Gizzard muscle, cardiac muscle, ovary, lung, testis, pancreas, kidney, liver and bone-marrow.

The growth activating effect of extracts of mitotically active tissues is less than that of extracts of some stable tissues (e. g. brain, heart, smooth muscle).

The extracts of spleen, an organ rich in leucocytes, are less active than extracts of some stable tissues.

It was shown that liver extract has a definite growth stimulating effect.

After finishing this work, a paper by Trowell and Willmer, 1939 (Studies on the growth of tissues in vitro. VI. The effects of some tissue extracts on the growth of periosteal fibroblasts, J. Exp. Biol., 16: 60) was brought to our notice. These authors, who used another method (determination of mitotic index) to test the stimulating effect on growth in vitro of extracts of adult organisms, came to conclusions which in many instances are similar to ours.

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BOOK REVIEW

Mathematics in Bacteriology, OTTO RAHN. III + 63 pp. $1.75. Burgess Pub. Co., Minneapolis, Minn. 1939.

This book is written as a text for students of biology and medicine who need to know the mathematical techniques of handling quantitative data such as are encountered in bacteriology. In an introductory chapter, analytical and graphical representations of simple cases of growth are given. Then follows an elementary presentation of the concepts of rate of growth and rate of change of rate of growth. Though methods of calculus are required, familiarity with calculus is not assumed and the subject is presented very simply.

Rahn prefers not to use the term growth in reference to populations since he strongly objects to the use of such phrases as "growth of *Pseudomonas fluorescens*" when the author means growth of a culture or population of *Pseudomonas fluorescens*. The term growth is restricted to indicate increase in size of individuals while in reference to increase in number of individuals of a population the term multiplication is used. Thus the rate of increase of a population is described by a multiplication rate or a multiplication constant if rate is constant. This constant is defined as the coefficient of time in an exponential equation to the base e. It seems unfortunate that the constant is defined in this way, for, as Rahn states, there is no simple interpretation of it. If the more natural base for this case, i. e., the multiplication per generation, had been used instead of e, then the coefficient of time would have been the potential growth rate in units of generations per unit time or, in other words, the reciprocal of the interfission period (e. g. Brown, 1939, and Brown, 1940). In addition to description of methods and determination of measures of population changes in unrestricted populations are found examples of analysis for rate of production of metabolites and of consumption of food and oxygen per cell in growing populations.

The equations for unlimited multiplication are considered to be approximations at all periods, for Rahn states that inhibiting factors which in later stages are so strong as to prevent multiplication and fermentation must exert their influence, though very weakly, even at the earlier stages. This may be true for many limiting factors, but an analysis in which factors limiting multiplication are considered to have threshold values would seem to be more general. Such an analysis will perhaps be the next stage in the evolution of methods for treating the phase of negative acceleration of multiplication rate in populations of microorganisms. The threshold may be very low for factors operating to limit most bacterial cultures, but in the case of limitation of certain protozoan cultures by decreased oxygen tension or exhaustion of food

(e. g., Phelps, 1935; Phelps, 1936, and Brown, 1940) the threshold may not be reached until after many generations at constant multiplication rate; and in a study of rate of respiration of certain bacteria it was found (Warburg and Kubowitz) that oxygen concentration does not become a limiting factor even though reduced to 10^{-5} normal.

The mathematical formulation of the action of factors limiting populations and population products used by Gause and others in which the rate of increase is considered proportional to the difference between the existing state and a maximum limiting state divided by the maximum is criticized by Rahn on the basis that this formulation fits the facts in such a limited number of cases. The example of alcoholic fermentation by yeast in which this formulation was applied by Gause is examined in detail and more correct methods of analysis, especially for determination of fermentation capacity, are developed.

The rate of death of populations of organisms under unfavorable environmental conditions is considered carefully, and methods are developed for determination of the effectiveness of disinfectants. These methods are based on death of microorganisms according to a logarithmic order of death. The derivation of the formula for the concentration exponent which is used as a measure of the effectiveness of antiseptics seemed somewhat shaky when first studied. This was due to the fact that it was not clear that the "endpoint" in a test for viable bacteria represents a more or less definite concentration (not zero) which may be used in equations for concentration of bacteria as a function of time whereas zero concentration could not have been used in these equations. At another point in the derivation the relationship that the rate of death in a bacterial culture is proportional to a power of the concentration of the disinfectant is used. The reasoning leading to this relationship is not given. It might have been as follows: The logarithmic order of death indicates death according to a monomolecular reaction which may be written

poison + bacterium$\rightleftharpoons$poison — bacterium complex$\rightarrow$dead bacterium

from which we would have

$$\frac{[\text{poison}]^{n}[\text{bacteria}]}{[\text{complex}]} = k$$

where n is the number of molecules of the poison required to form the complex. The rate of death of the culture would then be proportional to

$$\frac{p}{k}[\text{poison}]^{n}[\text{bacteria}]$$

where p is the rate for the monomolecular reaction (complex$\rightarrow$dead bacterium). The reviewer is not familiar with work in the field of

evaluation of disinfectants but would surely expect the factor $\frac{p}{k}$ to be of great significance for comparison of poisons that act through a monomolecular type of reaction. Rahn discusses no measure but the concentration exponent.

The important subject of statistics in bacteriology is treated briefly. The sections on measures of dispersion seem rather confusing since the definitions are for dispersion of individual measures and the formulae are for the probable error of the mean. The appropriate statistical techniques for estimation of cell concentration by dilution tests and for estimation of the correlation of properties of bacterial strains are well given.

This book contains many sets of illustrative data and references to other actual data that may be used in problems. This extensive inclusion of data and references to data is found since the book is designed to be used as a text in a course in which the student learns by solving problems. The concluding chapter is devoted to an excellent discussion of graphical representation of data and interpretation of graphs.

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